Design and optimization of a novel top-lit gas-lift bioreactor for industrial CO_2 mitigation and microalgae-sourced biodiesel production

by

Nekoo Seyed Hosseini

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy (PhD) in Natural Resources Engineering

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Design and optimization of a novel top-lit gas-lift bioreactor for industrial CO_2 mitigation and microalgae-sourced biodiesel production
Name of Candidate Nom du candidat	Seyed Hosseini, Nekoo
Degree Diplôme	Doctor of Philosophy
Department/Program Département/Programme	Natural Resources Engineering
Date of Defence Date de la soutenance	March 24, 2017

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. JA Scott
(Co-Supervisor/Co-Directeur(trice) de thèse)

Dr. Helen Shang
(Co-Supervisor/Co-directrice de thèse)

Dr. Gregory Ross
(Committee member/Membre du comité)

Dr. Junfeng Zhang
(Committee member/Membre du comité)

Dr. Zisheng Zhang
(External Examiner/Examinateur externe)

Dr. Carita Lanner
(Internal Examiner/Examinateur interne)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. David Lesbarrères
Monsieur David Lesbarrères
Dean, Faculty of Graduate Studies
Doyen, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

Abstract

Mitigation of CO_2 in industrial off-gasses by sparging the gas through photosynthetic microalgae bioreactors is an attractive concept. The goal is for the CO_2 to be consumed by the microalgae as a nutrient, which in turn produces lipids suitable for conversion into biodiesel, as well as other value-added bioproducts such as Omega-3 fatty acids and antioxidants.

Open systems are considered the most economic outdoor, large-scale cultivation option but have large land space requirements due to their shallow depths (15-35 cm). Consequently, finding sufficient space to locate them close to off-gas sources on industrial sites can be a significant challenge. Shallow depths are also likely to result in low uptake of CO_2 and consequently reduced biomass productivity due to short gas residence times in the culture medium.

In order to obtain longer gas/liquid transfer times, as well as greater per area productivity, the tanks through which the off-gas is sparged must be as deep as possible. However, to make the tanks deeper and avoid the costs associated with sub-surface artificial lighting, the issue is how to ensure the microalgae receive adequate light exposure. We have, therefore, looked for a novel method for increasing the depth of the tanks through which the off-gas is sparged. To achieve this, we have investigated the use of a gas-lift circulating system in a deep top-lit open bioreactor. In addition to providing CO_2, the sparged gas also provides continual vertical circulation of the microalgae to ensure good mixing and an adequate light/dark cycle. Compared to existing shallow open systems, the

results obtained showed comparable biomass productivity per unit volume, but importantly around three-times higher biomass productivity per unit area occupied by the bioreactor. The lipid productivity was also increased due to light and hydrodynamic stresses.

In order to enhance further light utilization efficiency in the deep cultivation bioreactor, the use of a novel non-energy-consuming light column was also evaluated. The results of using the light column showed a 33% increase in areal biomass productivity and a 16% increase in areal lipid production.

The proposed design and developed models can be easily translated into larger scale, on-site production facilities in industrial sectors emitting off-gas. The carbon capturing properties of microalgae can, therefore, help reduce industrial carbon dioxide emissions, whilst at the same time producing biodiesel from the resulting lipids.

Keywords

Microalgae, CO_2, off-gas, Biodiesel, Top-lit gas-lift bioreactor, Energy, Optimization

Acknowledgments

First, I would like to express my appreciation and gratitude to my supervisor Dr. Ashley Scott. It has been an honor to be his student and I appreciate his contributions of time, ideas, and funding to make my PhD experience productive and stimulating. His advice and inspiration on both research, as well as my life have been priceless. I could not have imagined having a better advisor and mentor for my PhD study.

Next, this journey would not have been possible without the support of my family. To my parents, thank you for believing in me and inspiring me to follow my dreams. Thank you for always being there for me, even through the tough times. To my brother, thank you for being my best friend and thank you for helping me grow.

I would also like to give special thanks to my thesis committee: Dr. Helen Shang, Dr. Greg Ross and Dr. Junfeng Zhang for their insight and encouragement.

Last but not least, I want to thank my friends and all the people who created such a great atmosphere in the lab and made this experience unforgettable.

Table of Contents

List of Figures

List of Tables

List of Publications

Seyed Hosseini N, Shang H, Ross GM, Scott JA (2016) Comparative analysis of top-lit bubble column and gas-lift bioreactors for microalgae-sourced biodiesel production. Energy Conversion and Management 130: 230-239. (Chapter Four)

Seyed Hosseini N, Shang H, Ross GM, Scott JA (2015) Microalgae cultivation in a novel top-lit gas-lift open bioreactor. Bioresource Technology 192: 432-440. (Chapter Three)

Eibl JK, Corcoran JD, Senhorinho GNA, Zhang K, **Seyed Hosseini N**, Marsden J, Laamanen CA, Scott JA, Ross GM (2014) Bioprospecting for acidophilic lipid-rich green microalgae isolated from abandoned mine site water bodies. AMB Express 4:7.

Seyed Hosseini N, Shang H, Scott JA (Submitted) Optimization of microalgae-sourced lipids for biodiesel production in a top-lit gas-lift bioreactor using response surface methodology - Energy. (Chapter Five)

Seyed Hosseini N, Shang H, Scott JA (Submitted). Increasing productivity of a top-lit deep open microalgal cultivation system for biodiesel production by using a light column – Biomass & Bioenergy. (Chapter Six)

Seyed Hosseini N, Shang H, Scott JA (Submitted) Biosequestration of industrial off-gas CO_2 and enhanced lipid productivity in open system microalgae cultivation for biodiesel production - Renewable and Sustainable Energy Reviews. (Chapter Two)

List of Presentations

The Bioeconomy Research and Innovation Forum (2016) - Guelph, ON, Canada
Poster presentation: Enhanced biodiesel production with a high rate top-lit gas-lift open bioreactor that can capture industrial off-gas CO_2

19th Conference on Process Integration, Modelling and Optimisation for Energy Saving and Pollution Reduction (2016) - Prague, Czech Republic
Oral presentation: An energy efficient top-lit gas-lift bioreactor for algal cultivation and CO_2 mitigation of industrial off-gas

24th European Biomass Conference & Exhibition (2016) – Amsterdam, Netherlands
Oral presentation: A high rate top-lit gas-lift open microalgae bioreactor that can utilize industrial off-gas to enhance productivity and allow a reduced footprint

12th World Congress on Industrial Biotechnology (2015) – Montreal, QC, Canada
Oral presentation: Development of a novel top-lit gas-lift bioreactor for algal cultivation and CO_2 mitigation of industrial off-gas

Mining and the Environment Conference (2015) – Sudbury, ON, Canada
Poster presentation: Biodiesel from microalgae bioreactors that utilize smelter off-gas CO_2

64th Canadian Chemical Engineering Conference (2014) – Niagara Falls, ON, Canada
Oral presentation: A novel microalgae bioreactor design that utilizes industrial off-gas for CO_2 capture to promote biofuel production

3rd International Conference on Algal Biomass, Biofuels and Bioproducts (2013) - Toronto, ON, Canada
Poster presentation: The use of a gas-lift bioreactor with industrial off-gas for microalgae production

2nd International Conference on Algal Biomass, Biofuels and Bioproducts (2012) - San Diego, USA
Poster presentation: The use of acidic industrial off-gasses for enhanced microalgae growth and lipid production

List of Abbreviations

Abbreviation	Description	Unit
A	Illuminated surface area	m^2
A_b	Free area between riser and downcomer	m^2
A_{550}	Absorbance at 550 nm	cm^{-1}
A_d	Cross-sectional area of downcomer	m^2
A_r	Cross-sectional area of riser	m^2
A_t	Total area occupied by bioreactor	m^2
c	Speed of light	ms^{-1}
C	Dissolved oxygen concentration at time t	mgL^{-1}
C_0	Initial dissolved oxygen concentration at time t_0	mgL^{-1}
C^*	Dissolved oxygen saturation concentration	mgL^{-1}
C_b	Dry biomass concentration	$g_{dw}L^{-1}$
C_{bmax}	Maximum dry biomass concentration	$g_{dw}L^{-1}$
C_{bi}	Initial dry biomass concentration	$g_{dw}L^{-1}$
C_{bf}	Final dry biomass concentration	$g_{dw}L^{-1}$
D	Desirability index	-
D_i	Draft tube diameter	m
D_o	Column diameter	m
D_{O2}	Oxygen diffusivity in water	m^2s^{-1}
D_{CO2}	Carbon dioxide diffusivity in water	m^2s^{-1}
d_H	Hydraulic diameter	m
d_B	Mean bubble diameter	m
d_t	Total bioreactor depth	m
E	Energy of photons	J
f	Darcy friction factor	-
g	Gravitational acceleration	ms^{-2}
h	Planck constant	Js
h_L	Unaerated height	m
h_D	Aerated height	m
h_r	Height of riser	m
h_d	Height of downcomer	m
I_i	Light intensity	$\mu molm^{-2}s^{-1}$
K_B	Friction loss coefficient	-
k_La	Volumetric mass transfer coefficient	s^{-1}
L_c	Length of circulation loop	m
P_a	Areal biomass productivity	$g_{dw}m^{-2}day^{-1}$
P_v	Volumetric biomass productivity	$g_{dw}L^{-1}day^{-1}$
P_L	Volumetric lipid production	$g_{Lipid}L^{-1}$
P_{La}	Areal lipid production	$g_{Lipid}m^{-2}$
P_{CO2}	Sequestration rate of CO_2 per unit area of bioreactor	$g_{CO2}m^{-2}day^{-1}$
P_G	Power input due to aeration	W
P_e	Energetic productivity	$g_{dw}W^{-1}day^{-1}$

t_c	Mean circulation time	s
t_l	Light period	s
Δt	Exponential growth phase	d
U_G	Superficial gas velocity	ms^{-1}
U_{Gd}	Superficial gas velocity in downcomer	ms^{-1}
U_{Gr}	Superficial gas velocity in riser	ms^{-1}
U_{Ld}	Superficial liquid velocity in downcomer	ms^{-1}
U_{Lr}	Superficial liquid velocity in riser	ms^{-1}
U_b	Mean bubble rise velocity	ms^{-1}
V_L	Average liquid circulation velocity	ms^{-1}
V_d	Actual liquid velocity in downcomer	ms^{-1}
V_r	Actual liquid velocity in riser	ms^{-1}
V_t	Total volume of bioreactor	m^3
W_i	Energy input	W
W_{Rr}	Energy loss due to wakes behind bubbles in riser	W
W_{Dd}	Energy loss due to stagnant gas in downcomer	W
W_{Fr}	Energy loss due to friction in riser	W
W_{Fd}	Energy loss due to friction in downcomer	W
W_B	Energy loss due to fluid turn-around at the bottom of bioreactor	W
W_l	Light power input	W
W_m	Mechanical power input	W
$X_{i,j}$	Independent factor	-
Y	Response	-
α	Constant coefficient	-
β_0	Independent coefficient	-
β_i	Linear coefficient	-
β_{ii}	Quadratic coefficient	-
β_{ij}	Interaction coefficient	-
ε	Overall gas hold-up	-
ε_d	Gas hold-up in downcomer	-
ε_r	Gas hold-up in riser	-
μ	Specific growth rate	day^{-1}
ρ_L	Culture density	kgm^{-3}
ρ_G	Density of mixture of air and CO_2	kgm^{-3}
θ_L	Lipid content (%)	-
ξ	Light fraction	-
λ	Light wavelength	nm
Ψ	Relationship between gas transfer and hold-up	s^{-1}

Chapter One: Introduction

1.1 Background

Anthropogenic greenhouse gas emissions have a significant effect on our climate at both a national and global scale. Fossil fuels are the primary cause of climate change, making it increasingly important to find new, alternative sources of energy as well as finding ways of mitigating current sources of carbon.

Microalgae has the ability to convert inorganic carbon into organic carbon in the form of cellular compounds such as lipids, proteins and carbohydrates. This is accomplished by assimilation of anthropogenic carbon dioxide (CO_2) as a source of carbon, nutrients such as nitrogen and phosphorus, and a photosynthetic reaction driven by light energy. The microalgal lipids containing triglycerides can be extracted using solvents and alcohol and converted into fatty acid methyl esters (biodiesel) and glycerol by transesterification (Rawat et al., 2013). Microalgae that mitigate CO_2, therefore, have the potential to replace current industrial consumption of diesel fuel.

For the transesterification reaction, alkali catalysts such as sodium hydroxide or potassium hydroxide are typically used to improve the efficiency. To increase the conversion rate, acidic or enzymatic catalysts can be also used (Chisti, 2007). However, Jazzar et al. (2015) reported direct transesterification of wet microalgae biomass under supercritical conditions without the use of a catalyst.

Due to the low CO_2 concentration of air (around 380 ppmv) and the high surface tension of water (Zimmerman et al., 2011), the diffusion of CO_2 from the atmosphere into a microalgal culture is limited which in turn inhibits the CO_2 fixation and microalgal growth rate. Providing supplemental carbon to the microalgal culture, therefore, enhances microalgal metabolism and growth. However, using inorganic carbon salts or compressed CO_2 gas in a large-scale cultivation facility contributes significantly to operational costs. Therefore, using the CO_2 contained in industrial off-gas, such as from the burning of fossil fuels, cement manufacture and ore smelting, is a desirable economic and environmental goal.

The reported concentration of CO_2 from various industrial sources varies. For example, 6-7% from nickel smelting, 9% from natural gas combustion, 10% from coal-fired power plants, 15-25% from cement production and 30% from steel production plant have been reported (Laamanen et al., 2014; Bounaceur et al., 2006).

Due to the high production costs of closed systems, open systems are considered the most economic route for large-scale production of microalgae biomass (Chisti, 2007), and account for approximately 95% of all systems worldwide (Mendoza et al., 2013). Richardson et al. (2012) reported that open system production costs were three-times lower than those of closed photobioreactors per unit volume of lipids.

Generally, microalgal open system production is found in regions with warmer temperatures to provide ideal growing conditions. To aid in the reduction of construction and energy costs, open systems are also typically built in the ground, utilizing sunlight as a means of eliminating artificial lighting. They usually operate at a water depth of 15-35

cm (Zhao and Su, 2014), which allows for acceptable levels of light attenuation and avoids the need for supplementary below surface lighting. It is also recommended to keep the algal culture density at less than 0.6 $g_{dw}L^{-1}$ in order to minimize the shadow effect (Tredici, 2003). If industrial off-gas is to be sparged in, shallow depths will, however, result in a short gas residence time in the culture medium, which in turn will reduce CO_2 transfer rate.

Due to their shallow operational depths, open systems have large surface areas. Therefore, finding adequate land area on an industrial site to locate them close to a CO_2 rich off-gas source is likely to prove to be challenging. If no close area is available, the costs of delivering the off-gas to a distant location can become prohibitively high (Putt et al., 2011).

Currently, paddlewheels that are used for mixing raceways provide a horizontal circulation velocity, but minimal vertical circulation (Prussi et al., 2014). Providing adequate horizontal and vertical mixing can enhance CO_2 distribution and assimilation, aiding in increasing CO_2 solubility in the culture. However, providing increased mechanical mixing with the aim of obtaining optimal mixing would increase operation costs and possibly damage or stress the microalgal cells (Michels et al., 2016; Chiaramonti et al., 2013).

Studies have been conducted to find ways of increasing biomass productivity and CO_2 mitigation in shallow open systems. Building single or multiple sumps across the raceways and incorporating baffles was proposed by Weissman et al. (1988) and De Godos et al. (2014). Circulating the microalgae culture in a gas-lift carbonation column to

increase the CO_2 absorptivity was studied by Putt et al. (2011). Replacing the paddlewheel mixing system with a gas-lift station was evaluated by Ketheesan and Nirmalakhandan (2012). All these studies showed promising results as related to CO_2 transfer efficiency, but a significant increase in areal productivity was not attained due to inefficient mixing and light exposure.

Increasing the depth of open systems will provide a longer gas-liquid contact time for enhanced CO_2 transfer and fixation efficiencies that could lead to higher biomass and lipid productivities. However, light penetration in deeper depths is restricted and sub-surface lighting would be required to provide enough light energy. In this study, we propose to address this issue by incorporating a gas-lift system in a deeper pond. The research aim is to improve productivity per unit area occupied by an open system by increasing the CO_2 transfer rate and light exposure of algal cells through continual vertical circulation. The novel design developed has the benefit of reducing the footprint on industrial sites.

1.2 Outline

In Chapter Two, the various microalgal cultivation systems are reviewed, along with methods for enhancing biodiesel production by modifying the operational factors. The advantages and disadvantages of using industrial off-gases as a source of carbon required for algal metabolism, and the means by which CO_2 is assimilated and cellular lipid is synthesized are also reviewed

In Chapter Three, the results of our research into growing microalgae in a one-meter deep, top-lit bioreactor which used a gas-lift system to enable the deeper than traditional open system depth, are reported.

Once the feasibility of the proposed design was validated, in Chapter Four we systematically compared two common gas-liquid contacting systems (bubble column and gas-lift bioreactors) regarding biomass and lipid productivities. Whilst the biomass productivity of the bubble column was higher, the gas-lift bioreactor showed greater lipid production. Since production of lipids plays the critical role as feedstock for biodiesel, further investigation was then conducted on maximizing lipid production in the gas-lift bioreactor.

In Chapter Five, the performance of the top-lit gas-lift bioreactor with various depths is, therefore, optimized in order to maximizing lipid production per occupied unit area. A statistical experimental design method was used to account for the interactive effects of operational parameters such as depth, sparging rate, initial biomass density, and CO_2 and nutrient concentrations. The optimal combination of operational parameters that maximize lipid productivity was found through a two-step, factor screening-optimization method and then verified with further experiments using the top-lit gas-lift bioreactor.

In Chapter Six, a novel non-energy-consuming light column was investigated as a means to increase the light utilization efficiency. The effectiveness of using this light column in the center of the top-lit gas-lift bioreactor was proven, and the operational factors involved in maximizing lipid production were screened and optimized through a response

surface methodology. Further experiments using the top-lit gas-lift bioreactor equipped with the light column were then used to verify the optimum configuration.

Finally, in Chapter Seven, a summary of the results of this study, the potentials and limitations of the proposed designs, as well as future prospects for translating these results into a pilot plant are discussed.

Chapter Two: Biosequestration of industrial off-gas CO_2 for enhanced lipid productivity in open microalgae cultivation systems

Paper #1 – Literature review, Renewable and Sustainable Energy Reviews (Submitted)

Nekoo Seyed Hosseini[1], Helen Shang[1] and John Ashley Scott[1,2*]

[1] Bharti School of Engineering, Laurentian University, Sudbury, ON, Canada

[2] Northern Ontario School of Medicine, Sudbury, ON, Canada

Abstract

Utilizing industrial off-gas CO_2 in open system photosynthetic microalgae cultivation is a biological means to mitigate greenhouse gas emissions. The captured CO_2 can also enhance production of microalgal lipids for conversion into biodiesel. However, environmental stressors such as temperature, pH, luminosity, salinity, metal toxicity, nutrient availability and shear stress can impact the CO_2 fixation process and lipid biosynthesis.

We discuss the mechanisms of carbon fixation and lipid synthesis in microalgal cells, commercial microalgae cultivation systems, and the limitations and potentials of utilizing industrial off-gases. The influences of operational and environmental factors on CO_2 sequestration rates and lipid production, as well as manipulative approaches for enhanced lipid production are reviewed.

Keywords: Microalgae, CO_2, off-gas, enhanced lipid, biodiesel, open system

2.1 Introduction

Sustainability is a primary principle in natural resource management that involves operational efficiency, minimization of environmental impact and socio-economic considerations (Singh and Sharma, 2012). Concerns about the lack of fossil fuels, variations in crude oil prices, greenhouse gas emissions and accelerated global warming show that continued reliance on fossil fuel energy resources is unsustainable.

Global warming and climate change are generally accepted as the consequence of increased greenhouse gas emissions. The temperature of the earth has increased by 0.85°C from 1880 to 2012, of which 0.6°C occurred in the past 30 years (Goeppert et al., 2012). It is further anticipated that the global temperature will rise to 5.8°C by 2100 (De Silva et al., 2015). The outcomes are expected to include melting polar ice and increased sea levels, changes in weather patterns leading to droughts and floods (De Silva et al., 2015), increased ocean acidity, species extinction and unbalanced biodiversity (Manzello et al., 2017; X. Wang et al., 2016; Anderson et al., 2016). These issues have led to a worldwide interest in anthropogenic CO_2 capture and mitigation methods, not least, due to the introduction of legislation restricting or capping industrial emissions (Lee et al., 2017; Yadav and Sen, 2017; Rubin et al., 2012).

Common CO_2 management methods include carbon capture and storage (CCS) through geo-sequestration and ocean-sequestration (Bielicki et al., 2016; Kim et al., 2016; Marshall, 2016), enhanced oil and gas recovery (EOR and EGR) (Shelton et al., 2016; Dai et al., 2016; Hasan et al., 2014), enhanced coal bed methane recovery (ECBM) (Ranathunga et al., 2017; Temizel et al., 2016; Laumb et al., 2013), chemical methods

(adsorption and absorption) (Ben-Mansour et al., 2016; Novek et al., 2016; Rubin et al., 2012), physical methods (cryogenic distillation or membrane filtration) (Maqsood et al., 2017; Mehrpooya et al., 2017; Sreedhar et al., 2017; Liu et al., 2016) and biological mitigation methods (Tsai et al., 2017; Kemper, 2015) through terrestrial plants, macro and microalgae, microbes and biochar (Hicks et al., 2017; Creamer et al., 2016; Mandal et al., 2016; Sondak et al., 2016).

However, the above existing methods do have disadvantages. For CCS these include high transportation costs and the possibility of reservoir leakage being released back to the atmosphere or oceans, which in turn could lead to ocean acidification (Lee et al., 2017; Szalaj et al., 2017; Zhang and Huisingh, 2017; Zwaan and Gerlagh, 2016). Chemical methods involving CO_2 separation from industrial off-gas by using solvents can suffer from evaporative losses, high energy requirements for solvent recycling and corrosion, leading to high operational costs (Budzianowski, 2017; Zhang et al., 2017; Cheah et al., 2016). Solid adsorbents such as alumina or zeolites that chemically adsorb CO_2 in active sites (Nikolaidis et al., 2017; Belmabkhout et al., 2016; Rubin et al., 2012) can lose efficiency very quickly with high temperatures, meaning that they are not suitable for many industrial off-gas streams (Hu et al., 2017; Madden et al., 2017; Zhang et al., 2016).

Cryogenic distillation, a physical method of carbon capture, is extremely energy intensive and is not considered economical for industrial practices (Song et al., 2017; Li et al., 2016; Goeppert et al., 2012). Membrane technology can be used to filter industrial off gases with a very high CO_2 concentration and they also foul easily causing large pressure drops (Alharthi et al., 2016; Geyer et al., 2016; Pires et al., 2012). Challenges of the EOR and EGR mitigation methods include high energy requirement for injecting pressurized

CO_2 in the enhanced oil and gas recovery methods, and leakage into the water aquifer (Dai et al., 2016; Marques and Pimentel, 2016).

All of these mitigation methods are based on using the CO_2 after separating it from the off-gas stream. Biological mitigation using microalgae can, however, capture CO_2 without the need to separate it from the off-gas. Microalgae in fresh and saline water use CO_2 as a carbon source and convert it into organic carbon for producing cellular compounds such as lipids, proteins and carbohydrates. The resulting biomass can be used as a feedstock for food, fuel, animal feed and value-added products (Anderson et al., 2016; Baicha et al., 2016; Moreira and Pires, 2016; Borowitzka, 2013a). Microalgae are also about 50 times more efficient at sequestering CO_2 than terrestrial plants due to faster growth rates (Klinthong, 2015; Bhola et al., 2014).

Microalgae, unicellular, filamentous or colonial, are simple in structure and energy is directed via photosynthesis into growth and reproduction without establishing or maintaining complex tissues and organs (S. K. Singh et al., 2016; Klinthong, 2015). In general, microalgae offer the prospect of high biomass yields without requiring any arable land and have also the potential to be cultivated in off-shore containment. Moreover, some microalgal species grow well in saline and wastewater, making them a more promising feedstock than terrestrial crops that rely on a supply of fresh water (Ansari et al., 2017; Yadav and Sen, 2017; Daroch et al., 2013).

The first mono-algal cultures, *Chlorella vulgaris,* were obtained by Beijernick (1890) and developed for studying plant physiology by Warburg in the early 1900's. Mass culture of

microalgae began to be a focus of research after 1948 at Stanford (USA), Essen (Germany) and Tokyo (Japan) (Borowitzka, 1999).

Large-scale commercial production of microalgae started in Japan with *Chlorella* in the 1960's, followed by *Spirulina* in Mexico and Thailand in the 1970's. Forty six of the worlds large-scale factories were producing greater than 12,000 kg of microalgae per year by 1980 (Borowitzka, 2013b). As a source of β-carotene, *Dunaliella salina* was the third microalgae plant established in Australia in 1986 (Spolaore et al., 2006). The commercial production of cyanobacteria (blue-green algae) started in India at about the same time. These plants were soon followed by other countries such as the USA and Israel. It should be noted that microalgae production plants around the world have been established in year-round warm climate areas, as opposed to regions such as North America and Northern Europe.

More recently, there has been significant interest in extracting lipids from microalgae and transesterifying them into biodiesel (Kumar et al., 2016; P. Singh et al., 2016). Most species of microalgae have a dry weight lipid content of 20-50% (Kumar et al., 2016; Rawat et al., 2013), whereas terrestrial crops (e.g. soy, canola, palm, corn or jatropha) have a lipid content of less than 5% (Ho et al., 2014; Chisti, 2008).

Despite many studies conducted on biological anthropogenic CO_2 mitigation through microalgae cultivation, there is a lack of reviews on large-scale cultivating facilities aimed at biodiesel production. This review summarizes, therefore, the limitations, potentials, and impacts of operational and environmental factors on CO_2 biosequestration and lipid production suitable for conversion to biodiesel.

2.2 CO_2 sequestration in microalgae

Biological carbon sequestration using microalgae has been more recently considered as a method to mitigate anthropogenic CO_2 through photosynthesis, whilst producing value added products. Sequestration of CO_2 in microalgae occurs in two steps: a carbon concentrating mechanism and a photosynthesis process.

2.2.1 Carbon concentrating mechanism

In microalgae, Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the main enzyme involved in catalyzing CO_2 fixation through the reductive pentose phosphate pathway (Hanson, 2016; Raven and Beardall, 2016). The first product of carbon fixation, 3-phosphoglyceric acid (3-PGA), is converted into essential elements of cells such as carbohydrates, lipids and amino acids (Meyer et al., 2016; Mondal et al., 2016).

Poor performance by RuBisCO due to a low affinity for CO_2, a tendency to react with O_2 and a low turnover rate results in energy consuming photorespiration, and a low CO_2 fixation rate (Kroth, 2015; Sage and Stata, 2015). Low diffusivity of CO_2 in water is also a limiting factor in RuBisCO enzymatic activity. Dissolved inorganic carbon in water can exist at equilibrium in forms of CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}, but only HCO_3^- and CO_2 are considered as substrates for RuBisCO (Raven and Beardall, 2016).

Bicarbonate (HCO_3^-) can be consumed by microalgae directly by cation exchange and active transport or indirectly through catalytic conversion into CO_2 and OH^- (Meyer and Griffiths, 2013). Enzymatic conversion of dissolved inorganic HCO_3^- into CO_2 is aided by carbonic anhydrase enzymes (CA). There are three types of carbonic anhydrase

enzymes: (i) Periplasmic Carbonic anhydrase (pCA) for keeping a balance between CO_2 and HCO_3^- and continuously supplying CO_2 to cells; (ii) Cytosolic Carbonic Anhydrase (cyCA) for accelerating the transport of CO_2 and HCO_3^- from the plasma membrane to chloroplasts; and (iii) chloroplast carbonic anhydrase (chCA) as the inorganic carbon transport system located on the chloroplast envelope that delivers HCO_3^- to the stroma (Badger and Price, 1994).

The process of increasing the CO_2 level at the RuBisCO active sites is known as the CO_2 concentrating mechanism (CCM), which increases CO_2 fixation and photosynthetic rates and inhibits photorespiration (Zhao and Su, 2014). However, the CO_2 concentrating mechanism was not detected in golden-brown algae, likely limiting the distribution of these algae to niches with adequate CO_2 availability (Kroth, 2015; Maberly et al., 2009). RuBisCO has been observed in major algal species in one or more crystal-like proteinaceous structures within plastids called pyrenoids (Raven and Beardall, 2016; Raven et al., 2012). Despite an unclear functionality of pyrenoids, Moroney and Ynalvez (2007) proposed that pyrenoids might play an important role in carbon concentrating mechanisms by separating RuBisCO from the CA in the stroma of the chloroplast (Kroth, 2015).

2.2.2 Photosynthesis process

In the process of photosynthesis, photoautotrophic microalgae use light energy in the active radiation range (400-700 nm) to convert CO_2 into glucose while releasing oxygen. This can be empirically presented as follows:

$$6\ H_2O + 6\ CO_2 \rightarrow C_6H_{12}O_6 + 6\ O_2 \quad (1)$$

This process occurs in two light dependent and light independent stages. First, in the light dependent stage, microalgal cells absorb and store light energy. Antenna complexes and carotenoids transfer the light energy to the photochemical reaction centers of P700 and P680 (part of photosystem I and II) on the thylakoid membrane of the chloroplast (Iverson, 2006). Molecules of ADP and $NADP^+$ are converted into energy carrying molecules of ATP and NADPH through an electron transport chain. Excited electrons are transferred to electron acceptors, leaving the reaction center in an oxidized state (Zhao and Su, 2014). Molecular oxygen is the product of this stage.

In the light independent stage, absorbed CO_2 along with ATP and NADPH molecules generated in the previous stage are converted to organic compounds through the Calvin-Bassham-Benson cycle, which is the essential pathway for photoautotrophic reactions (Katiyar et al., 2016; Kroth, 2015).

2.3 Microalgae cultivation system

For mass-production of microalgae, there are different systems available. These can be classified as open systems, controlled closed systems called photobioreactors (PBR) and hybrid systems. However, around 95% of worldwide commercial microalgae biomass production is currently carried out in open systems (Mendoza et al., 2013).

Open cultivation systems are left open to the atmosphere and growing conditions are rarely controlled. Open systems include tanks, circular ponds with rotating arms to mix the culture, raceway ponds with paddlewheels to circulate the culture and cascade systems with baffles. They are simple and cheap to construct, maintain and operate, but potential problems include risk of contamination, poor mixing, dark zones, water loss due

to evaporation, poor mass transfer efficiency and inefficient use of CO_2 (Goli et al., 2016; Ullah et al., 2015). All of these can reduce the productivity of microalgae cultivation and CO_2 mitigation.

Raceway ponds should theoretically have production levels of 50–60 $g_{dw}m^{-2}day^{-1}$, but in practice, productivities of even 10–20 $g_{dw}m^{-2}day^{-1}$ are difficult to achieve (Seyed Hosseini et al., 2015; De Godos et al., 2014; Christenson and Sims, 2011). Due to light penetration and scattering of light by microalgae cells, the common large-scale production systems in practice usually operate at a water depth of 20-50 cm (Baicha et al., 2016; Kumar et al., 2016).

Another major drawback is large land space requirements (typically 0.2-0.5 ha) for cultivation due to their shallow depth requirement (Zhao and Su, 2014; Vasumathi et al., 2012). Furthermore, production in open ponds depends on the local climate due to a lack of temperature control and the cost of water (Pires et al., 2012). Smaller scale open system facilities can be covered with transparent material to prevent contamination and loss of water and CO_2, to improve productivity (Kumar et al., 2015).

The reason that most large-scale facilities are operated as open systems is the high production cost of closed systems. In a study conducted by Richardson et al. (2012), the average production cost of one gallon of lipids in an open system was around 60% cheaper than that in closed photobioreactors. In a study by Chisti (2013), the maximum production cost of dry biomass for microalgae with a minimum lipid content of 40% needs to be $0.25/kg to be considered a successful, large-scale biodiesel production facility if enough and a feasible supply of CO_2 is provided.

The Earthrise company in California, USA claims to be the largest outdoor open cultivation facility for *Spirulina* production (www.earthrise.com). It is located in a desert, covers 0.5 ha and operates for seven months of the year (Kumar et al., 2015). The Muradel Demonstration plant in Whyalla, Australia grows microalgae in 0.4 ha paddle wheel open raceways (www.muradel.com). Other examples of commercial scale open microalgae cultivation facilities include euglena in Japan (www.euglena.jp), Hainan-DIC in China (www.hidic.com.cn), Algatech in the Czech Republic (www.alga.cz) and Algae system in the USA (www.algaesystems.com).

There are also hybrid systems that combine an open system with a closed photobioreactor to improve biomass productivity, reduce the high cost of a completely closed photobioreactor operation and lower the risk of contamination associated with open systems. In the first stage, microalgae are cultivated in a closed photobioreactor up to a desired density. For the second stage, a dense microalgal culture is transferred to an open system to scale-up biomass production. The two-stage cultivation strategy in the hybrid system can be also used for high lipid production suitable for conversion into biodiesel (Narala et al., 2016; Singh and Sharma, 2012). First, microalgae grow under ideal nutrient rich conditions in a photobioreactor, and the dense culture is transferred to a low nutrient environment in an open system. Nutrient deficiency is known as a stressor to induce lipid synthesis in microalgae (P. Singh et al., 2016; Chen et al., 2015).

2.4 CO_2 biosequestration from industrial off-gases in open systems

Despite the attraction of using microalgae for both carbon sequestration and as a biodiesel source, there are currently limitations to this being an economically viable

option. For microalgae cultivation, in addition to the CO_2 supply, the species type, temperature, media pH, nutrients supplied, light intensity and distribution, dissolved oxygen, water source and mixing must all be considered because they affect capital and maintenance costs (Goli et al., 2016; Narala et al., 2016; Rawat et al., 2013).

2.4.1 CO_2 content

Microalgal CO_2 utilization requires two stages: absorption of CO_2 from the gas phase by mass transfer and chemical reaction and fixation of CO_2 by photosynthesis (Vasumathi et al., 2012). Diffusion of CO_2 into the media from the atmosphere above an open tank hinders microalgal growth due to a low presence of CO_2 in air (around 380 ppmv) and the high surface tension of water (Seyed Hosseini et al., 2015). Providing an additional supply of carbon can increase biomass production (Zhao and Su, 2014), but adding purchased inorganic carbon, such as compressed CO_2 or bicarbonate salts has a large impact on production costs. Li et al. (2013) stated that the cost of the carbon source contributes up to 27% towards production costs. Therefore, the use of "free" CO_2 from industrial processes off-gas is seen as an appealing economic option. CO_2 concentration from natural gas combustion, coal-fired power plants, and steel production have been reported as 9%, 10% and 30%, respectively (Seyed Hosseini et al., 2015; Bounaceur et al., 2006). Cement production has been reported to release 15–25% (Lara-Gil et al., 2016; Olofsson et al., 2015) and nickel smelting, 6–7% CO_2 in their off-gas streams (Laamanen et al., 2014).

There have been many studies into the optimum concentration of CO_2 supplied to microalgae for optimum biomass and lipid production (Tsai et al., 2017; Ramanan et al.,

2010). Low concentrations of CO_2 can be a limiting factor in algal growth, which can be also hindered when the CO_2 concentration is too high, or if off-gas is sparged in continuously (Lam et al., 2012). Excessive exposure to CO_2 is thought to limit growth as a consequence of a reduction in the capacity of microalgal cells to uptake carbon due to denatured cellular enzymes (S. K. Singh et al., 2016; Kumar et al., 2011).

2.4.2 Depth

Open commercial raceways lit by sunlight typically operate at a water depth of 15–35 cm (Zhao and Su, 2014) due to light attenuation (self-shading). The depth of open systems with sparged in CO_2 has a direct impact on CO_2 and light utilization efficiencies due to short gas residence times. Weissman et al. (1988) reported an 80–90% loss of CO_2 to the atmosphere because of the shallow depth. Poor mixing due to shallow depths also creates dark zones that lead to inefficient light utilization (Prussi et al., 2014). Increasing mechanical energy to achieve homogeneous conditions would, however, negatively affect operation costs and potentially damage or stress microalgal cells (Michels et al., 2016; Chiaramonti et al., 2013). In open systems, these limitations resulted in areal biomass productivity of 20 $g_{dw}m^{-2}day^{-1}$ on average as opposed to 45-60 $g_{dw}m^{-2}day^{-1}$ in closed system (Seyed Hosseini et al., 2015; Handler et al., 2012). Sutherland et al. (2014) found greater areal biomass productivity with greater depths when comparing 40 cm to 20 cm depths, due to an increased CO_2 absorption rate and better nutrient removal by the microalgal cells. Moreover, in regions with high-temperature variations, greater depths provide a more consistent environment for cell growth resulting in an enhanced CO_2 sequestration rate and areal productivity (Slegers et al., 2013).

2.4.3 Location

The shallow depth of open systems results in a large footprint. When industrial off-gas is to be utilized as a source of carbon for the photosynthesis process, finding the required large area on, or close to the industrial off-gas source may be a challenge. Other parameters to be considered in selecting the industrial site location are lithological characteristics, slope of the land, water availability and quality, climate conditions including precipitation levels, seasonal temperature range, sunlight radiation, and cloud cover (Andersen, 2005). The National Algal Biofuels Technology Roadmap (U.S. Department of Energy (DOE), 2010) suggests areas, where rainfall intensity is higher than 100 cmy^{-1}, are suitable for microalgae production facilities. Bennett et al. (2014) similarly stated that rainfall intensity in the range of 150-300 cmy^{-1} would be the base case condition. The effect of dilution, however, due to a high precipitation rate should be considered as it affects the cost of harvesting due to higher volume to be processed downstream (Barros et al., 2015; Laamanen et al., 2016). Land with no more than a 2 to 5 % slope is also suggested due to lower excavation cost (Bennett et al., 2014).

2.4.4 Nutrients

Depending on the microalgal species requirements, the availability of essential nutrient sources is another factor influencing CO_2 sequestration and biomass production. Nitrogen and phosphorus are the most important nutrients for microalgal growth, but trace substances such as metals (Mg, Ca, Mn, Zn, Cu, and Mb) and vitamins (B_1, B_{12}, and H) are also typically added for effective cultivation (Kumar et al., 2016; S. K. Singh et al., 2016). Ammonium and phosphate compounds are typically used to supply the nitrogen

and phosphorus required for metabolism and the carbon fixation process (X. Wang et al., 2016; W. Y. Cheah et al., 2015). In open systems, nutrient concentrations will fluctuate due to precipitation levels and evaporative losses, which can have a negative impact on microalgal cell metabolism and growth due to cellular ionic and osmotic stresses (Meijer et al., 2016; Chen et al., 2015). Some microalgae species, however, have an osmoregulatory mechanism to manage the change in nutrient concentrations (Pancha et al., 2015; Osundeko et al., 2013). Monitoring the salinity concentration during the cultivation cycle and access to low-cost fresh water and nutrient sources are keys to enhancing biomass productivity. Assimilation of organic compounds and nutrients from wastewater and sewage for microalgal growth is also a promising approach in wastewater and sewage treatment plants. The ratios of N/P and C/N in the wastewater profile have an important role in CO_2 biosequestration and biomass production (W. Zhou et al., 2014).

2.4.5 Mixing

Good mixing of the culture media prevents cells from settling, improves the CO_2 transfer rate, avoids nutrient concentration gradients, increases light utilization efficiency, eliminates accumulated dissolved oxygen, thereby lowers toxicity, and homogenizes temperature and pH. Mixing in open systems is often provided by mechanical stirring such as a rotating arm, paddlewheel, and baffles, or in conjunction with CO_2 delivering systems such as porous gas diffusers (plate or tube) and membrane sparged devices (Kumar et al., 2015; Greenwell et al., 2010). Paddlewheel technology, which is relatively simple and inexpensive, is widely used for mixing raceways and provides a horizontal liquid velocity of 0.1–0.3 ms^{-1} (Yadav and Sen, 2017; De Godos et al., 2014), but limited vertical agitation. As CO_2 solubility is low, providing adequate horizontal and vertical

mixing is necessary for enhanced CO_2 distribution and assimilation. However, input of mechanical energy is limited due to energy costs and risk of cell damage or stress by high local shear intensities (Michels et al., 2016; Markou and Nerantzis, 2013). Therefore, use of gas-liquid contacting devices with low shear stress is a better option in mixing microalgal culture. The geometry of off-gas diffusers can also have an impact on CO_2 transfer rates, as smaller pore sizes will form smaller bubble sizes, which result in higher mass transfer coefficients (Seyed Hosseini et al., 2016; Knežević and Povrenović, 2015). However, greater loss of CO_2 to the atmosphere due to bursting bubbles at the surface and biofouling of the diffuser or membrane (Merriman et al., 2014) are other issues that may reduce the CO_2 sequestration rate.

Various designs have been proposed to increase productivity and CO_2 capture efficiency in traditional shallow open systems. Placing diffusers at the bottom of single or multiple sumps with/without baffles (De Godos et al., 2014; Bao et al., 2012) has been demonstrated to provide higher gas transfer rates. Cheng et al. (2016) reported a 29% increase in biomass yield by using up-down chute baffles. Putt et al. (2011) used a bubble column to carbonate the culture before entering the raceway. An airlift-driven design was proposed by Ketheesan and Nirmalakhandan (2012) as a means of replacing paddlewheels. Du et al. (2012) showed a higher CO_2 injection efficiency using a venturi injector over a conventional diffuser system. A CO_2 supplying device consisting of a trap container with a clapboard divider in the middle and diffusers at the bottom, was fixed at the bottom of a pond and shown to enhance CO_2 absorptivity (Su et al., 2008).

2.4.6 Temperature

The optimal temperature varies among species and also depends on environmental conditions. Certain microalgal species have been able to grow at temperatures of up to 60°C (Kumar et al., 2011), but optimal growth temperatures of 15-26°C have been reported for most species, with maximum cell densities obtained at 23°C (Kumar et al., 2010). Higher temperatures reduce CO_2 solubility in the culture, which results in bonding of the RuBisCO enzyme with O_2 instead of CO_2. This causes photorespiration and reduction of the CO_2 fixation rate up to 30% (Zeng et al., 2011). Low temperatures also inhibit RuBisCO activity and reduce the CO_2 bioconversion and photosynthetic rates (Zhao and Su, 2014).

Large-scale open systems are typically located in regions with warm temperatures, which can cause considerable evaporative losses (Chisti, 2007). These regions are often semi-arid and suffer from a lack of freshwater. Whereas regions that are abundant in freshwater generally experience seasonal cold climates, and are consequently not currently considered for outdoor microalgal production. Industrial off-gases released to the environment can contain, however, a significant amount of waste heat that could be utilized to maintain the temperature of open ponds in cold climate regions (Laamanen et al., 2014; Shang and Scott, 2011).

In regions or seasons (e.g., summer) where off-gas waste heat does not need to be captured for maintaining pond temperature, it can be utilized for drying wet biomass in downstream processes (Laamanen et al., 2014). Cooling treatments can be also applied to the off-gas before being bubbled in the cultivation system. The high costs of cooling and

scrubbing the off-gas will, however, contribute to the production costs of biomass and lipid production. The use of thermo-tolerant species such as *Chlorella* sp. is an alternative option that avoids high cooling treatment costs (Razzak et al., 2013).

2.4.7 pH

The favorable pH for most freshwater microalgae species is neutral, and a pH of 8 is ideal for marine microalgae (González-López et al., 2012). There is, however, a complex relationship between CO_2 concentration and pH in microalgal cultures, owing to the underlying chemical equilibrium among chemical species such as: CO_2, H_2CO_3, HCO_3^- and CO_3^{2-} (Kumar et al., 2010):

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+ \leftrightarrows CO_3^{2-} + 2\ H^+ \quad (2)$$

In the absence of supplemental CO_2, pH is elevated to around 8 due to activity of the carbonic anhydrase (CA) enzyme, transferring hydroxide ions to outside the cell (Kumar and Das, 2012). The absorption rate of CO_2 increases at higher pH due to reaction between hydroxyl ions and CO_2 to produce bicarbonate (González-López et al., 2012). The CO_2 content of many sources of off-gas at 5-10 % (v/v) will reduce the pH of the microalgal culture to 5.5 (Chen et al., 2015) due to the CO_2 equilibrium achieved between consumption and supply rates. Using a buffer such as sodium hydroxide or calcium carbonate to adjust pH to the microalgal strain dependent optimal level when industrial off-gas is being utilized, can enhance the CO_2 fixation rate (Bartley et al., 2014; Kroumov et al., 2016).

2.4.8 Light

Light as the energy source for metabolism is probably the most important factor affecting carbon fixation. Microalgae can utilize energy available in the wavelength range of 400-700 nm, which is referred to as photosynthetically active radiation (PAR). Light energy is represented in terms of photon flux density (PFD) and is often measured in Lux. The photosynthetic rates are determined by the average PFD to which individual cells are exposed (Vasumathi et al., 2012). Growth kinetics of microalgae increase with light intensity up to a critical point when photo-inhibition occurs, due to damage to the microalgae's photosystems and the pigments involved in photosynthesis (Kumar and Das, 2012; Pires et al., 2012). Deactivated photosystems within microalgae require time to recover, which is why alternating periods of light and dark were introduced (Abu-Ghosh et al., 2016). A light/dark cycle is required, therefore, to achieve high productivity and reduced photo damage effects.

In open systems, the light intensity attenuation and the light/dark zone cell exposure frequency are affected by depth, biomass density, and mixing patterns. In current large-scale ponds, the maximum depth of operation is 50 cm (Baicha et al., 2016; Ketheesan and Nirmalakhandan, 2012), although the depth for efficient penetration of sunlight is typically only 15-20 cm (Alaswad et al., 2015; De Godos et al., 2014). Under these conditions, the culture concentration should be maintained at less than 0.6 $g_{dw}L^{-1}$ to reduce self-shading and allow light to penetrate deeper (Tredici, 2003). A high light/dark cycle frequency has been recommended for algal culture when the growth is light-limited (Ketheesan and Nirmalakhandan, 2011).

Light intensity for CO_2 bioconversion has been reported to be in the range of 10 to 400 $\mu molm^{-2}s^{-1}$. For outdoor cultivation, a minimum sunlight energy of 4.65 $kWhm^{-2}d^{-1}$ (around 880 $\mu molm^{-2}s^{-1}$) is required to reach a sustainable growth rate (Bennett et al., 2014). A higher light intensity may be required for dense cultures to allow deeper light penetration (Zeng et al., 2011).

2.4.9 SO_x and NO_x

In addition to CO_2, industrial off-gases usually contain potentially toxic compounds, such as sulfur oxides (SO_x) and nitrous oxides (NO_x) that can have negative impacts on the growth rate of microalgae (Lam et al., 2012). SO_2 concentrations above 50 ppm inhibit microalgal growth due to the creation of a low pH environment (Van Den Hende et al., 2012). The pH may reduce to 2.5 due to SO_2 hydrolysis and the formation of HSO_3^-, SO_3^{2-} and SO_4^{2-} in the culture when 250 ppm of SO_2 is present in the off-gas (Lam et al., 2012). Most algal species cannot survive in pH lower than 4 (Zhao and Su, 2014). Desulfurization processes such as dry and wet methods using CaO sorbent and limestone-gypsum may need to be considered for removing SO_2 before sparging off-gas into the cultivation system (Lee et al., 2005).

Unlike SO_2, high concentration of NO_x in the off-gas does not inhibit algal growth rate. In the culture media, NO is oxidized to NO_2^- in the presence of oxygen, which reduces its pH. However, Yoshihara et al. (1996) observed that concentrations of NO_x up to 300 ppm can be absorbed by microalgae cells as a nitrogen source during the exponential growth phase. In general, nitrogen in the form of N_2, NO, NO_2^-, NO_3^- and NH_4^+ can be absorbed by algal cells for their cell metabolism (Van Den Hende et al., 2012).

2.4.10 Heavy metals

The presence of heavy metals in industrial off-gases can also have inhibitory effects on microalgal growth. Napan et al. (2015) stated that a combined concentration of ten heavy metals (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, and Zn) in ash from coal-based off-gas, higher than 17.5 μM reduced the CO_2 fixation rate, biomass production, and lipid synthesis. Off-gases from metal industries, such as smelting, are usually processed through an electrostatic precipitator (ESP) to precipitate and remove particles that contains heavy metals (Shang and Scott, 2011; Van Den Hende et al., 2012). However, power plant off-gases usually contain low metal concentrations at about 0.05 ppm (Matsumoto et al., 1997), which may make it a better option for microalgae cultivation.

2.4.11 Oxygen concentration

Carbon fixation by microalgae is accompanied by oxygen production as a by-product during the photosynthesis reaction. The dissolved oxygen (DO) concentration in the culture media increases as growth rate increases. Jiménez et al. (2003) reported that DO concentrations higher than 25 ppm would inhibit CO_2 sequestration. A high DO activates the oxygenase activity of the RuBisCO enzyme rather than carboxylase due to the lower RuBisCO affinity constant of oxygen compared to CO_2 (Hanson, 2016). The oxygenase reaction uses notable amounts of cellular energy and consequently results in loss of CO_2 (Kumar et al., 2015). Therefore, enough turbulence should be provided to prevent oxygen accumulation even if sparging CO_2 into the culture is not required.

2.5 Biodiesel from Microalgae

More than 70% of microalgae lipid content is made up of triglycerides (Olofsson et al., 2012). These triglycerides can be solvent extracted and converted into biodiesel by transesterification in the presence of alcohol (Rawat et al., 2013). Fatty acid methyl esters (FAMEs) and glycerol are the products of this chemical reaction. To improve the efficiency of the reaction, alkali catalysts such as sodium hydroxide or potassium hydroxide are usually involved in the reaction. Acidic or enzymatic catalysts can also be used to increase the conversion rate (Chisti, 2007). Direct transesterification of wet microalgae under supercritical conditions without the presence of catalyst has also been reported (Jazzar et al., 2015).

Microalgal cells have various compositions of carbohydrates, lipids, and proteins, depending on cultivation conditions, species and growth phase. There are reports that certain strains of microalgae can produce lipid contents up to 85% on a dry weight basis (Wu et al., 2013; Shang et al., 2010). Therefore, changing environmental conditions including temperature, pH, CO_2 concentration, nutrients, illumination, and mixing as well as harvesting time, can be applied for manipulating the quality and quantity of lipid production.

2.5.1 Enhanced Biodiesel production

Large-scale potential of biodiesel production needs an accumulation of high lipid content in microalgal cells. Under optimal species related cultivation environments, microalgae cells typically take the biosynthesis pathway to synthesize fatty acids for use as membrane components such as glycolipid or phospholipids (Markou and Nerantzis,

2013; Hu et al., 2008). Under stressed growth conditions, microalgae may, however, modify their biosynthetic pathways to increase the formation of triacylglycerol (TAG), which is accumulated in cytosolic lipid bodies as storage for both carbon and energy (Sibi et al., 2016; Roleda et al., 2013; Hu et al., 2008).

Fatty acids are the main units of cellular lipids that can be stored as membrane glycerolipids, storage components and energy sources including TAGs. An acetyl-CoA (coenzyme A) is one of the main components in the formation of fatty acids in the chloroplast (Katiyar et al., 2016). In the fatty acids synthetic pathway, an acetyl-CoA carboxylase (ACCase) enzyme produces malonyl-CoA from acetyl-CoA and bicarbonate. In the next step, glycerolipids are synthesized in the endoplasmic reticulum from these components (Hu et al., 2008). Under unfavorable conditions, TAGs are synthesized by consecutive acylation of glycerol-3-phosphate (G3P) with three acyl-Co-A in the endoplasmic reticulum (Katiyar et al., 2016).

While the selection of high-lipid producing strains is initially important in the production of biodiesel, microalgae have shown increased lipid production along with potential alterations in lipid quality, when exposed to physiological stressors (Eibl et al., 2014; Widjaja et al., 2009). Environmental stressors can improve lipid quality and quantity by altering biomolecular pathways (Cao et al., 2014; Roleda et al., 2013; Mata et al., 2010).

As growth and stress are seen as opposing processes, the application of stressors during the microalgal growth phase may significantly reduce biomass production (Venkata Mohan and Devi, 2014). It is, therefore, generally proposed that microalgal cultivation incorporates a two-stage approach (Aléman-Nava et al., 2017). In the first stage,

conditions for optimal biomass growth are maintained and in the second stage, enhanced lipid accumulation is created as a result of an environmental stress exposure. This two-stage cultivation strategy provides optimized biomass and lipid accumulation (Narala et al., 2016; Cao et al., 2014; Xia et al., 2014; Roleda et al., 2013). Santos et al. (2014) for example, demonstrated an increased yield of lipids in optimally grown *Neochloris oleoabundans* that was subsequently exposed to a high pH stress. Doan and Obbard (2015) noted a 2.3-fold increase in total fatty acid content with the application of a 16-day stress phase by adding sodium acetate after an 11-day optimal growth stage. Total lipid content of *Nannochloropsis* sp. was reached to 40% by stressing cells with low temperature (10°C) and low light (30 $\mu molm^{-2}s^{-1}$) at the second stage (Mitra et al., 2015).

2.5.1.1 CO_2 concentration and pH

The CO_2 concentration of the sparged in off-gas has an impact on the quantity and quality of the microalgae lipid content. Yusof et al. (2011) reported fatty acid composition variations of *Chlorella vulgaris* by varying CO_2 concentration in outdoor cultures. Vidyashankar et al. (2013) reported an increase in lipid content from 10 to 20% for *S. dimorphus* while growing under 2% (v/v) CO_2 in air. A similar observation of a lipid content increase with higher CO_2 concentration was reported by Tang et al. (2011), where they found that lipid content increased by 9% and 27% when bubbling in air with 5% and 10% CO_2, respectively. Xia et al. (2013) investigated the effect of CO_2 content on lipid productivity of *Chlorella* sp. in a range of 5–15% and found maximal productivities at 10% CO_2.

High concentrations of CO_2, however, result in a decrease in pH of the culture media due to conversion of unused CO_2 to carbonic acid (H_2CO_3). The decreased pH can lead to restricted microalgae growth, despite a greater supply of carbon, due to inhibited RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) activity in the CO_2 concentrating mechanism (Zhao and Su, 2014). Tatsuzawa et al. (1996) studied the impact of pH on fatty acid production and showed a higher relative percentage of triacylglycerol to the total lipid content when growing *Chlamydomonas reinharadtii* isolated from an acidic volcanic lake in pH 1 compared to higher pH values of 3, 6 and 7. Cultivation under a low pH environment also resulted in high lipid content of *Scenedesmus* sp. in bodies of water isolated from an abandoned mine site (Eibl et al., 2014).

2.5.1.2 Nutrients

Nutrient deficiency is known as a trigger to induce lipid synthesis (Chen et al., 2015; Santos et al., 2014; Roleda et al., 2013; Ruangsomboon et al., 2013; Ruangsomboon, 2012). Lack of nitrogen (Wang et al., 2015; Liu et al., 2012; Feng et al., 2012), phosphorus (Mandal and Mallick, 2009; Ruangsomboon et al., 2013; Wu et al., 2013), salinity (Venkata Mohan and Devi, 2014; Bartley et al., 2013; Kan et al., 2012), silicon (Griffiths and Harrison, 2009) and iron (Ruangsomboon et al., 2013; Yeesang and Cheirsilp, 2011; Liu et al., 2008) have all been reported to enhance lipid production. Roleda et al. (2013) found an increase in lipid productivity of various *Chlorella* sp. due to a 5-12 day period of growth under optimized conditions followed by a 12-19 day stress period using nutrient deficiency and sub-optimal temperature. Takagi et al. (2000) found a change in lipid composition from fatty acids to triglycerides as a result of limited

nitrogen supply and Cakmak et al. (2012) showed an increase in total neutral lipids in response to nitrogen starvation. Khozin-Goldberg and Cohen (2006) reported an 83% decrease in phospholipid content and a six-fold increase in triacylglycerol (TAG) due to phosphate deprivation. It has, however, been demonstrated that lipid accumulation due to nitrogen starvation is more efficient than phosphorus starvation (Ruangsomboon et al., 2013; Feng et al., 2012; Mandal and Mallick, 2009).

Low nitrogen concentration results in a structural reduction of a subunit of the RuBisCO enzyme. This change in RuBisCO leads to an accumulation of acetyl-CoA carboxylase (ACCase) and increased enzymatic activity during the carbon fixation process. Therefore, instead of being used for protein synthesis, the CO_2 source is utilized for synthesis of storage molecules such as triglyceride (Katiyar et al., 2016).

2.5.1.3 Temperature

Temperature has a significant impact on the quantity and quality of lipids produced, which in turn, determines both the physical and chemical properties of the resulting biodiesel (Yang et al., 2016; Wei et al., 2015; Roleda et al., 2013; Sayegh and Montagnes, 2011). However, there appears to be no consistency in the reported responses of microalgae to temperature variations. Xin et al. (2011) for example, studied the growth and lipid accumulation properties of *Scenedesmus* sp. over a temperature range of 10–30°C and stated that the optimal temperature to produce lipid was 20°C. An increase in total lipid content of many microalgae species with increasing temperature has been reported (Sayegh and Montagnes, 2011). However, total lipid content of *Chlorella sorokiniana* increased after the temperature was dropped from 26°C down to 18°C (Y.

Wang et al., 2016). Řezanka et al. (2017) reported an increase in fatty acids content of *Monoraphidium* sp. when cultivated outdoors in early winter conditions of Central Europe with an average temperature of 10°C. Conversely, a decrease in triacylglycerol content of microalgae *Nitzschia* sp. was reported at a reduced temperature of 15°C (Chen et al., 2008). Wei et al. (2015) reported an increase in saturated fatty acids by increasing the temperature to 35°C. Cao et al. (2014) showed that both low temperature (10°C) and high temperature (45°C) enhanced lipid accumulation in *C. minutissima*.

In a two-stage cultivation strategy, cultivation and stress temperatures have an influence on both oxidative stability and low-temperature properties of the produced biodiesel, based on the accumulation of either saturated or unsaturated lipids (Xin et al., 2011; Hu et al., 2008). Sibi et al. (2016) reported an increase in the unsaturated to saturated fatty acids ratio with decreased temperatures.

2.5.1.4 Mixing

As explained in section 2.4.5, a high level of turbulent mixing created by sparging in CO_2-containing off-gas can aid in microalgal growth. However, due to shear stress, an excessive degree of mixing can damage the cells. This would result in growth impairment, the redirection of carbon flux from the starch to the lipid synthesis pathway, and an increase in lipid content (S. K. Singh et al., 2016; Mata et al., 2010). Mixing stress due to an increased gas to liquid ratio has been demonstrated to have a positive effect on growth and lipid formation of algal cells (Song et al., 2014). With high mixing stress, a decrease in the fatty acid methyl ester content per lipid obtained has also been

reported. This was explained due to limited triacylglycerol biosynthesis by the glycerol pathway from high gas to liquid ratios (Olofsson et al., 2012; Song et al., 2014).

2.5.1.5 Light

Light is one of the important triggers needed to induce lipid synthesis in microalgal cells. The light source (Yan et al., 2016), intensity (He et al., 2015a; Přibyl et al., 2016), spectra (Vadiveloo et al., 2015), light-dark photoperiods (W. Y. Cheah et al., 2015), frequency (Maroneze et al., 2016), and light-exposed surface area (W. Zhou et al., 2014) have all been reported to have a significant impact. They have a particular impact on the microalgae biomass formation rate and the biochemical content, including lipids, carbohydrates, proteins, vitamins, pigments and antioxidants (Wu et al., 2015).

Baer et al. (2016) studied the effects of spectral light quality on *Chlamydomonas reinhaedtti* growth and found a composition of α_{red}:α_{green}:α_{blue} of 80:10:10 resulted in the highest biomass productivity. Ra et al. (2016) achieved the highest lipid content of 52% for *Nannochloropsis salina* under narrow band green LED light stress. He et al. (2015a) reported a suitable fatty acid profile for biodiesel and an increase in lipid accumulation in *Chlorella* sp. and *Monoraphidium* sp. by increasing the light intensity from 40 $\mu molm^{-2}s^{-1}$ up to 400 $\mu molm^{-2}s^{-1}$. Wu et al. (2015) also obtained a similar result in *Gracilaria lemaneiformis* when they increased the light intensity from 20 $\mu molm^{-2}s^{-1}$ to 200 $\mu molm^{-2}s^{-1}$. Das et al. (2011) studied variations in the fatty acid content and composition of *Nannochloropsis* sp. exposed to different light intensities and wavelengths (red, green, blue and white). The maximum fatty acid methyl esters (FAME) content was achieved under green LED (550 nm). However, the highest volumetric FAME yield was obtained

under blue LED (470 nm) due to the highest biomass productivity at this wavelength. Cheirsilp and Torpee (2012) showed enhanced lipid production by stepwise increasing light intensity in fed-batch cultivation settings. Maroneze et al. (2016) reported an increase in lipid productivity when the photoperiod was changed from 12:12 to 24:0 (light:dark hours). However, the study of Atta et al. (2013) stated that the lipid content of *Chlorella vulgaris* decreased by 13% when increasing the photoperiod from a 12 to 16 hours light period at the same light intensity. This decrease in lipid content at higher luminance exposure was attributed to higher chloroplastidial activity to prevent photochemical cell damage.

Light stress caused by flashing light has been shown to stimulate microalgal lipid synthesis (Abu-Ghosh et al., 2016). Yoshioka et al. (2012) also reported enhanced lipid yield under a flashing blue light effect. Choi et al. (2015) achieved the highest microalgal growth under a flashing time stress of 10 times per min, while maximum volumetric FAME production was reached under a flashing time stress of 5 times per min. However, there are studies showing a decrease in the lipid content of microalgal cells cultivated under flashing light at different frequencies compared to continuous light (Combe et al., 2015; Sforza et al., 2012).

2.5.1.6 Salinity

Salinity has an impact on the physiological and biochemical mechanisms of microalgal growth and development (Venkata Mohan and Devi, 2014). A high salinity environment causes osmotic stress, leading to an alteration of metabolism to adapt to the stressful condition (Kan et al., 2012). An increase in intercellular lipid synthesis and change of

fatty acid metabolism have been observed as defensive mechanisms against salt injury (Asulabh et al., 2012; Ruangsomboon, 2012). Rao et al. (2007) showed a two-fold increase in fatty acid concentration of *Botryyococcus braunii* with a change of salinity. Bartley et al. (2013) also reported a significant increase in triglyceride content of *Nannochloropsis salina* and a decrease in membrane lipid content with increased salinity.

However, excessive salt concentration may inhibit CO_2 fixation and biomass production of microalgae and consequently reduces lipid productivity. Takagi et al. (2006) for example, reported elevated lipid content of up to 70% in *Dunaliella* cells, but hindered cell growth in response to increased NaCl concentration. In order to overcome this issue, a two-stage cultivation strategy has been studied where addition of salt occurred after the culture had reached an optimum biomass level. Various sodium salts have been investigated and shown to induce lipid accumulation in green microalgae through out a two-stage cultivation process (Xia et al., 2014; Gardner et al., 2012; Heredia-Arroyo et al., 2011). Venkata Mohan and Devi (2014) also showed a variation of the fatty acid profile due to salinity stress under a two-stage cultivation process.

A high saline environment can also inhibit growth of competitors and predators such as non-target microalgae and grazers (Bartley et al., 2013). This is an important factor in open system cultivation facilities as it may not only help with the induction of lipid synthesis, but also impede undesirable organisms.

2.5.1.7 Metal concentration

Metal exposure is known to affect lipid build-up in microalgal cells and trace levels of metals are commonly found in off-gases (V. Singh et al., 2016). Yeesang and Cheirsilp

(2011) and Liu et al. (2008) reported higher lipid storage in *Botrycoccus* sp. and *Chlorella* sp. when growing under higher iron levels of 0.037 and 0.012 mM, respectively. Ruangsomboon et al. (2013) also showed an increase in lipid content of microalgae by increasing the iron concentration from 9 to 45 mgL^{-1}.

In *Scenedesmus obliquus*, calcium limitation and magnesium supplementation induced lipid accumulation by 53% and 55% of dry cell weight respectively (Esakkimuthu et al., 2016). An enhanced lipid content of *Chlorella* sp. was observed by increasing the concentration of copper from 0.5 to 4 mgL^{-1} (Sibi et al., 2014). Lipid synthesis of *Euglena gracilis* was inhibited by a high chromium concentration, and the ratio between saturated and unsaturated fatty acids increased with an increase in chromium concentration (Rocchetta et al., 2006).

2.5.1.8 Oxidative stress

Excessive formation and accumulation of intercellular reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and the hydroxyl radical, results in cellular damage through oxidation of cellular components (Chokshi et al., 2015; Osundeko et al., 2013). Many environmental stresses stimulate the oxidative stress condition, which in turn induces lipid synthesis and changes the lipid profile of microalgal cells (Machado and Soares, 2016; Pancha et al., 2015; Zhang et al., 2013). Microalgal cells can, however, mediate anti-oxidative defense through the activities of ROS detoxifying enzymes such as Ascorbate peroxidases (APX) that detoxifies peroxides (Osundeko et al., 2014; Tanaka et al., 2011). There is an apparent increase in microalgal lipid content due to oxidative

stresses (Kang et al., 2014; Yilancioglu et al., 2014), but, a systematic explanation for this outcome needs further study (Sibi et al., 2016).

2.5.1.9 Growth phase

The various growth phases during microalgae cultivation result in different compositions of the cells. During the exponential growth phase, there is a higher proportion of protein and, therefore, harvesting microalgae during this phase results in biomass that can be useful for nutritional purposes. Late harvesting at the end of the stationary phase results, due to nutrient deficiency, in a high lipid content that is suitable for conversion into biodiesel (Alaswad et al., 2015). Lowrey et al. (2015) reported a 31% lipid content in *N. oculata* during the exponential growth phase and 40% and 50% at the beginning and end of the stationary phase, respectively. Therefore, selecting the right harvesting time can be helpful in achieving the desired product.

2.6 Conclusion

Mass production of microalgae has emerged as a potential means to mitigate carbon dioxide (CO_2) emitted in off-gasses from various industrial sectors (e.g., cement manufacture, energy generation and ore smelting) in order to reduce greenhouse gas emissions. Microalgae are also a promising feedstock for biodiesel production, due to their ability to synthesize higher quantities of fatty acids and triacylglycerols, especially under stressed conditions, which are suitable for transesterification into biodiesel.

There is a range of cultivation parameters and environmental factors influencing microalgal CO_2 biosequestration rates, biomass growth and lipid synthesis. Manipulating

single or multiple parameters in cultivation facilities can be used, therefore, to both enhance CO_2 mitigation rates and lipid production. Achieving a proper balance between the CO_2 absorption rate, biomass production, and lipid synthesis is of great importance to the economics and scalability of biodiesel production. Optimizing this balance is an area worthy of further investigation, especially in terms of finding the best combination of growth and stressing parameters.

Chapter Three: Microalgae cultivation in a novel top-lit gas-lift open bioreactor

Paper #2 – Original Research, Bioresource Technology, 192 (2015) 432-440

Nekoo Seyed Hosseini[1], Helen Shang[1], Gregory M. Ross[2], and John A. Scott[1,2*]

[1]Bharti School of Engineering, Laurentian University, Sudbury, ON, Canada

[2]Northern Ontario School of Medicine, Sudbury, ON, Canada

Abstract

Mitigation of CO_2 in industrial off-gas through its uptake by microalgae bioreactors is an attractive concept in the quest for biodiesel. Open systems are considered as an economic large-scale cultivation option but have large land requirements due to their shallow operational depths of around 0.3 m. Finding sufficient space to locate them close to fixed off-gas sources on industrial sites is, therefore, a challenge. The aim of this work was to investigate a top-lit open bioreactor that uses a gas-lift system to enable deeper ponds, thereby significantly reducing the footprint. Growth of *Scenedesmus* sp. in a one-meter deep, gas-lift bioreactor by sparged with 6% CO_2-enhanced air was evaluated. The results gave comparable volumetric biomass productivity (0.06 $g_{dw}L^{-1}day^{-1}$), but around three-times higher areal productivity (60.0 $g_{dw}m^{-2}day^{-1}$) than reported for traditional raceways. The energetic productivity (0.41 $g_{dw}W^{-1}day^{-1}$) obtained was comparable or greater than reported for photobioreactors and the lipid content of the *Scenedesmus* sp. was increased by 27% with an enhanced level of CO_2 in the sparging gas.

Keywords: Microalgae, Top-lit gas-lift bioreactor, Open pond, Off-gas, Areal productivity

3.1 Introduction

Due to dwindling reserves of fossil fuels and the impact on the global environment that their consumption can cause, alternative energy sources are needed. Specific species of microalgae with high photosynthetic rates and lipid content have the potential to provide one such alternative liquid fuel, biodiesel (Chisti, 2007). Furthermore, through microalgae photosynthesis there is opportunity to fix anthropogenic carbon dioxide (CO_2) from industrial point sources to both improve productivity and mitigate greenhouse gas emissions.

The diffusion of CO_2 from the atmosphere into a microalgal culture limits biomass productivity due to the low CO_2 content of air (around 380 ppmv) and the high surface tension of water (Zimmerman et al., 2011). Enhancing the supply of algae accessible carbon could, therefore, improve biomass density (Zhao and Su, 2014) and hence the economics of biodiesel production. However, adding inorganic carbon as bicarbonate salts or compressed CO_2 involves a relatively large cost. Therefore, the use of CO_2 bearing off-gas from industrial process is seen as an attractive economic option. Bounaceur et al. (2006) reported the CO_2 concentration in the off-gas from natural gas combustion, coal-fired power plants, steel and iron production as 9%, 10%, and 30%, respectively. The CO_2 content of cement production off-gas has been reported as 15-25% and as 6-7% in smelter furnace off-gas (Laamanen et al., 2014). In addition, industrial off-gases released to the environment contain significant amount of waste heat that could be utilized to maintain the temperature of open ponds in cold climate regions (Shang and Scott, 2011).

Among various methods proposed for large-scale cultivation of microalgae (Zhao and Su, 2014) open oval raceways circulated by use of a paddlewheel are currently the most economic option for commercial scale production due to relatively low capital, maintenance and operation costs (Chisti, 2007). They are typically located in regions with warm temperature and also have intensive light intensities, which can cause considerable evaporation losses and possible photoinhibition (Chisti, 2007). These regions are often semi-arid and suffer from lack of freshwater. Whereas regions that are rich in freshwater generally experience seasonal cold climates and consequently are not considered for outdoor algal production unless sources of "free" heat, such as that contained within off-gas can be utilized (Shang and Scott, 2011).

Open commercial raceways lit by sunlight only typically have large surface areas (e.g., 978m^2/pond (Chisti, 2007)), but only operate at a water depth of 15-35 cm (Zhao and Su, 2014). Providing an appropriate large land space close to a fixed off-gas source may, therefore, prove difficult on an industrial site and distribution piping costs to transfer off-gas to remote algal farms will contribute significantly in the cost of cultivation (Putt et al., 2011). If the costs of supplementary below the surface lighting is to be avoided, then the depth is limited due to restricted sunlight penetration. However, if off-gas is to be bubbled through, the shallow depth is likely to lead to reduced CO_2 (and where applicable heat) transfer due to a short residence time. Weissman et al. (1988) reported 80-90% loss of CO_2 to the atmosphere. Overall, these limitations have led to low utilization of CO_2 and areal productivity (20 $g_{dw}m^{-2}day^{-1}$) in traditional raceway ponds.

Alternative designs have been presented to increase productivity and CO_2 capture efficiency in traditional raceways such as: using single or multiple sumps with/without

baffles (De Godos et al., 2014; Weissman et al., 1988), a carbonation column system to circulate the culture through an absorption column (Putt et al., 2011), a carbon supplying device fixed at the bottom of the pond (Su et al., 2008), and an airlift-driven raceway design as a replacement of the current paddlewheel-driven design (Ketheesan and Nirmalakhandan, 2012). Although proposed configurations have provided enhanced CO_2 transfer efficiency, the reported areal productivities were not significantly improved.

Poor mixing, dark zones and inefficient light utilization are other factors inhibiting the productivity of open systems. Paddlewheel technology, which is relatively simple and inexpensive, is currently used for mixing raceways and provides a 0.1-0.3 ms^{-1} horizontal liquid velocity, but limited vertical agitation. Increasing mechanical energy to achieve good turbulent mixing would markedly affect operation costs as well as potentially damage or stress microalgal cells.

A more desirable approach to utilizing CO_2 from off-gas to achieve longer gas-liquid transfer times, as well as providing greater per area (areal) productivity on industrial sites would be to have deeper ponds. In order to make the ponds deeper and avoid the cost of artificial lighting, gas-lift systems could be employed to provide vertical circulation of the microalgae. Gas-lift colums have gained acceptance for gas-liquid contacting applications in bioprocessing due to efficient mixing with low stress, high volumetric gas transfer and a lack of microbial growth on the walls (Kumar and Das, 2012).

There have been studies on deep vertical photobioreactors for algal biomass product (Luo and Al-Dahhan, 2012; Barbosa et al., 2003a), but they have been generally restricted to enclosed bioreactors that are lit from the sides and the top. The use of a gas-lift system in

large-scale open ponds has not, however, been widely studied. Furthermore, if relatively cost-effective open system designs are to be used with off-gas bubbled through, lighting will be restricted to solar radiation from the top. Otherwise the additional expense of installing, running and maintaining sub-surface lighting will be needed.

In this study, we have evaluated the feasibility of cultivating microalgae in a one-meter deep top-lit open bioreactor coupled with a gas-lift system. The volumetric and areal productivies, CO_2 sequestration rate and power requirement of the proposed configuration are reported and compared with traditional raceways and photobioreactors. In addition, the imapct on lipid content of the *Scenedesmus* sp. was assessed.

3.2 Material and Methods

3.2.1 Microalgae and culture medium

Scenedesmus dimorphus obtained from the University of Texas, Austin collection (1,237 UTEX collection) was used for all experiments. The *Scenedesmus* species has been shown to out produce other microalgae, such as *Chlorella* sp. and *Chlorococcum* sp., with respect to percentage lipid content produced (Vidyashankar et al., 2013) and ability to grow under a wide range of CO_2 concentrations. The seed culture was grown in freshwater Bold's Basal medium (Andersen, 2005) in covered 180 L (120 x 30 x 50 cm) glass tanks at 22±2°C under cool white fluorescent light (approximately 60 $\mu molm^{-2}s^{-1}$) and on a 12 hours light/dark photoperiod. The cultures were continuously sparged with air for agitation and supplied with fresh Bold's Basal growth media every three weeks.

3.2.2 Laboratory scale bioreactor

The gas-lift bioreactors were constructed out of clear plexiglas tube with an internal diameter of 20 cm and wall thickness of 5 mm (Figure 3.1). For operation as a gas-lift reactor, a concentric draft tube of 13 cm internal diameter and 80 cm height was inserted 5 cm above the sparger to avoid high stress spots (Luo and Al-Dahhan, 2012). The orientation of the two vertical tubes forms the riser (the inner column) and the downcomer (the annulus) areas of the bioreactor. The ratio of cross sectional area of downcomer to riser and diameter of draft tube to outer column (A_d/A_r and D_D/D_C) were 1.37 and 0.65, respectively, which was within the range of values used by other studies (Luo and Al-Dahhan, 2012). The columns have side ports at 5 cm and 50 cm from the base for taking samples. Air was bubbled into the columns at a controlled flow rate and constant pressure through a ceramic sparger with an outer diameter of 4 inch and mean pore size of 15 microns (Refracton Technologies Crop., NY, USA). Carbon dioxide from a gas cylinder was mixed with air to achieve a 3% and 6% CO_2 mix content and the flow monitored by rotameters (Omega Engineering Ltd., QC, Canada).

On the premise that an industrial raceway type design is to be employed, (sun)light will only come from the surface. Therefore, for this work the bioreactors sides were covered with two layers of white sheet to increase reflectance and simulate the plastic-lined open pond and a black sheet on top to prevent any light penetration from the sides. The only light source was from directly above the liquid surface. This light was supplied by a circular 90W grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) on a 12 hours light/dark photoperiod. The intensity at the surface of the culture was approximately 1050 $\mu molm^{-2}s^{-1}$. Make up water was added

daily to keep the volume of culture constant due to evaporation loss. The bioreactors were operated at 22±2°C.

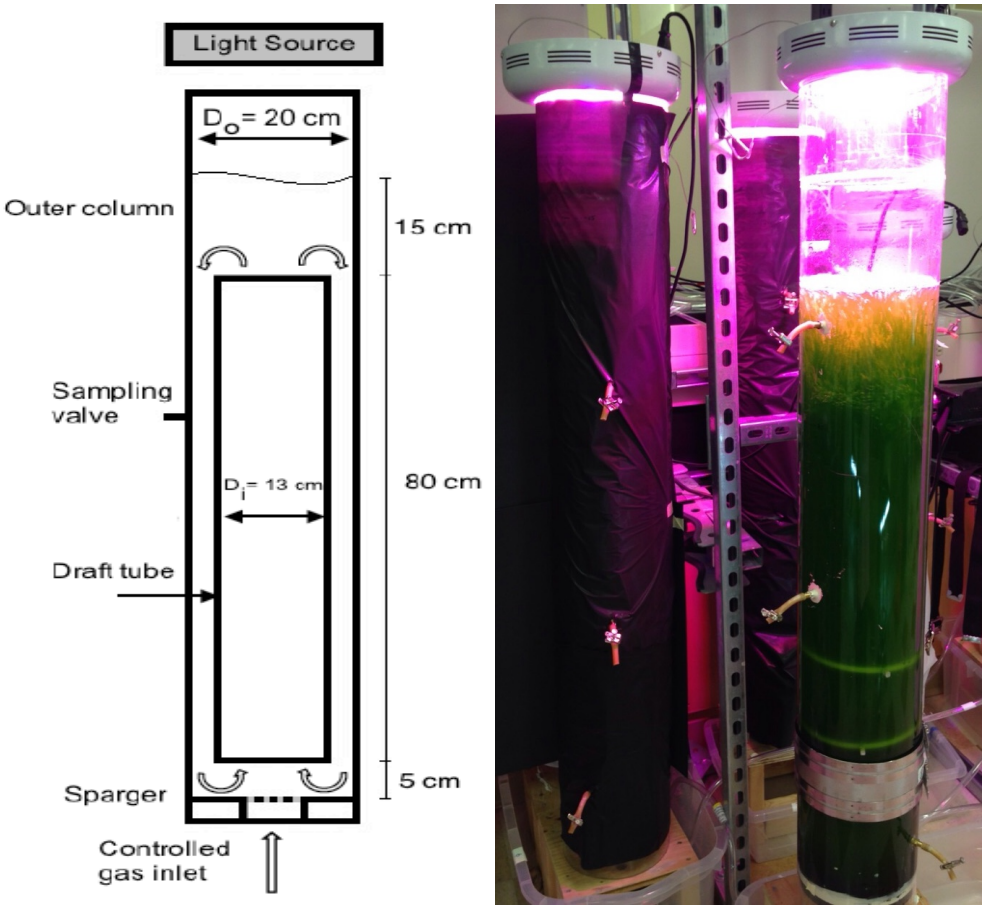


Figure 3.1: Schematic diagram and experimental set-up of the top-lit gas-lift bioreactor (right-hand column is uncovered for display purposes)

3.2.3 Circulation time and light fraction

In typical internal loop gas-lift bioreactors with an evenly distributed surrounding external light source for microalgae cultivation (Luo and Al-Dahhan, 2012), the downcomer comprises the light zone and receives the highest light intensity. The riser is considered a dark zone with a photosynthetic flux density less than 50 $\mu molm^{-2}s^{-1}$ due to

algal self shading (Barbosa et al., 2003b). However, if a gas-lift system is to be used in a buried deep pond, the only light source available is sunlight from the top, which causes an alternative orientation of light and dark regions. The surface of culture receives the highest light intensity, which decreases exponentially with depth. Measurements of the light intensity transition from the light to dark region were carried out using a light meter (LI-250 A, LI-COR Biosciences, NE, USA) equipped with a quantum sensor (LI-193SA, LI-COR Biosciences, NE, USA) at increments of known distances below the surface. An intensity of 50 $\mu molm^{-2}s^{-1}$ was considered the minimum for the light zone (Barbosa et al., 2003b).

Mean circulation time (t_c) was calculated by measuring the time taken for a 5mm colored tracer bead (Engineering Laboratories, NJ, USA) with the same density as water to circulate one cycle through a chosen horizontal reference plane in the column. The light period (t_l) was measured by the same method, but only the time in the light region was recorded. Then light fraction (ξ) was calculated at different gas flow rate as follow:

$$\xi = t_l/t_c \quad (1)$$

3.2.4 Growth and CO_2 sequestration determination

The growth of microalgae in the bioreactors was monitored by measuring optical density. 1 mL samples of culture were taken from the bioreactor with no dilution and placed in a quartz cuvette. The sample was then analyzed using a spectrophotometer (UV-1700 UV-VIS, Shimadzu, Tokyo, Japan) at a wavelength of 550 nm, which is outside the range of absorbance by Chlorophyll pigments (Andersen, 2005).

Dry weight of biomass, C_b ($g_{dw}L^{-1}$), was measured by vacuum filtering of 100 ml of algal culture using glass microfiber filter paper (Grade 151, Ahlstrom Filtration LLC, PA, USA), followed by oven-drying over night at 60°C.

Specific growth rate, μ (day^{-1}), was calculated by measuring the initial (C_{bi}) and final (C_{bf}) dry biomass concentration ($g_{dw}L^{-1}$), during the logarithmic growth phase (day) as follows (Andersen, 2005):

$$\mu = \frac{\ln(C_{bf}/C_{bi})}{\Delta t} \quad (2)$$

Volumetric productivity, P_v ($g_{dw}L^{-1}day^{-1}$), was calculated from the following equation over cultivation time, t (day) (Tang et al., 2011):

$$P_v = \frac{C_{bf}-C_{bi}}{\Delta t} \quad (3)$$

Areal productivity, P_a ($g_{dw}m^{-2}day^{-1}$), was calculated based on the total volume and surface area occupied by the bioreactor.

Assuming 50% of the dry weight of microalgae biomass is carbon, the theoretical required mass of CO_2 for the cultivation of 1 g dry weight of microalgae is 0.5(44/12)=1.83 g (Weissman et al., 1988), where 44 and 12 are the molecular weights of CO_2 and carbon, respectively. The sequestration rate of CO_2 per unit area of algal culture, P_{CO2} ($g_{CO2}m^{-2}day^{-1}$), during the cultivation period could be then estimated.

3.2.5 Growth kinetics

A logistic equation is considered to present the growth profile of the microalgae in terms of dry biomass concentration as a function of time. This describes lag, exponential and stationary phases of the culture (Kumar and Das, 2012).

$$\frac{dC_b}{dt} = \mu C_b\left(1 - \frac{C_b}{C_{bmax}}\right) \quad (4)$$

where C_b is the dry biomass concentration ($g_{dw}L^{-1}$), μ is the specific growth rate (day^{-1}), C_{bmax} is the maximum dry biomass concentration ($g_{dw}L^{-1}$). By integration and rearranging, we can get:

$$C_b = \frac{C_{bmax}}{1+\left(\frac{C_{bmax}}{C_{b0}}-1\right)\exp(-\mu t)} \quad (5)$$

where C_{b0} is the initial dry biomass concentration ($g_{dw}L^{-1}$) and C_{bmax} and μ are estimated by fitting the model with experimental data using Matlab (R2013a).

3.2.6 CO_2 mass transfer

Dynamic gassing-in and gassing-out methods (Sánchez Mirón et al., 2000) were used to evaluate the volumetric mass transfer coefficient (K_La) of CO_2 in the gas-lift bioreactor based on penetration theory and a measured oxygen absorption rate by using Eq. (6):

$$\frac{dC}{dt} = K_La(C^* - C) \quad (6)$$

Dissolved oxygen (DO) concentration was measured with DO probes (ORION 083010MD, Thermo Fisher Scientific Inc., MA, USA) located at the center of the riser

and the downcomer. The column was first bubbled with nitrogen gas till the DO concentration dropped to less than 5% of air saturation. After stopping the nitrogen flow and allowing the bubbles to leave the liquid, air at 9 $Lmin^{-1}$ was sparged and the DO concentration rise monitored until it reached 100% saturation. By integration of Eq. (6), the K_La of O_2 can be then calculated as the slope of the linear Eq. (7) (Sánchez Mirón et al., 2000):

$$\ln\left(\frac{C^*-C_0}{C^*-C}\right) = K_La\,(t-t_0) \quad (7)$$

where C* is the DO saturation concentration, C_0 is the initial DO concentration at time t_0 and C is the DO concentration at time t.

K_La of CO_2 was then calculated from the K_La of O_2 according to penetration theory (Kumar and Das, 2012):

$$K_La\,(CO_2) = \sqrt{\frac{D_{CO_2}}{D_{O_2}}}\,K_La(O_2) \quad (8)$$

where D_{CO2} and D_{O2} are the diffusion coefficients of CO_2 and O_2 in water, respectively.

3.2.7 Lipid Characteristics

Total lipid content as $g_{lipid}/g_{dw,biomass}$ was performed by lipid extraction using a modification of the method described by Folch et al. (1957). Freeze-dried algae samples were mixed with 1.2 mL of chloroform:methanol (2:1 v/v) in a centrifuge tube, and then sonicated using a Sonic Dismembrator Model 500 (Fisher Scientific, Ottawa, Canada) for 30 min. The sonicated samples were centrifuged for 15 min at 4750 rpm (Allegra X-15R

Centrifuge, Beckman, Palo Alto, CA, USA) and the solvent removed to a weighed vial. Extraction from the biomass was repeated three times and the resulting solvent was combined. The combined extract was dried by vacuum drier and the mass of the lipid determined gravimetrically.

3.2.8 Power requirements

Power input to the system includes the mechanical energy of gas aeration and light energy.

Mechanical power input for the gas-lift reactor, W_m (W), due to isothermal expansion of gas as it moves up and by neglecting kinetic energy of the injected gas to the bioreactor was calculated as follows (Chisti, 1989):

$$W_m = \frac{U_{Gr}\rho_l g A_t h_L}{1+\frac{A_d}{A_r}} \quad (9)$$

where U_{Gr} is superficial gas velocity in the riser (ms^{-1}), ρ_L is the culture density (kgm^{-3}), assumed to be the density of water and g is gravitational acceleration (ms^{-2}) . A_t is the entire cross-sectional area (m^2), and $h_{L,}$the unaerated height of the column (m) emphasizes the impact of water column height on the power requirements.

Light power input, W_l (W), was estimated by using measurement of light intensity, I_i ($\mu molm^{-2}s^{-1}$), at the surface of culture:

$$W_l = 0.5\alpha I_i A \quad (10)$$

where A (m^2) is illuminated surface area and α is a constant coefficient that depends on lighting conditions (as described in 3.2.2). In this study, α was calculated as 0.19 by determining the energy of photons at the relevant wavelengths (Walker et al., 2011):

$$E = \sum \frac{hc}{\lambda} \quad (11)$$

where h is Planck's constant, c is the speed of light and λ is the wavelength.

Therefore, energetic productivity, P_e in terms of daily biomass productivity per unit power input ($g_{dw}W^{-1}day^{-1}$), could be calculated from:

$$P_e = \frac{P_a A}{W_m + W_l} \quad (12)$$

3.3 Results and Discussion

3.3.1 Circulation time and light fraction variation

Light penetration with depth is a key factor in the growth of microalgae and it can be impacted on by algal density (self-shading) and gas flow rate (presence of bubbles). Light intensity readings were taken at 5 cm increments from the surface into the culture at gas volumetric flow rates in the range of 1-16 $Lmin^{-1}$. Figure 3.2 shows typical decreasing light intensity profiles with water and with an algal culture (biomass density of 0.1 $g_{dw}L^{-1}$) at a gas flow rate of 9 $Lmin^{-1}$. As expected, the results show a significantly greater rate of decline with an algal culture present due to self-shading. The attenuation coefficients were 0.15 cm^{-1} and 0.03 cm^{-1} for the algal culture and water, respectively.

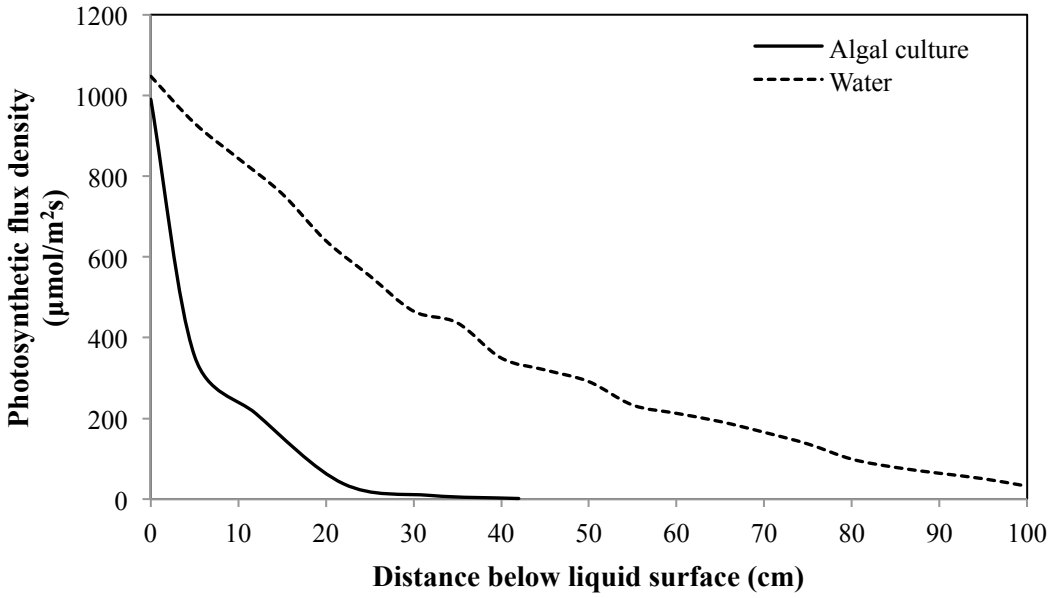


Figure 3.2: Light intensity measured at 5 cm depth increments (9 L/min gas flow rate)

In a gas-lift bioreactor, algal cells can take advantage of intermittent lighting by cycling through the light and dark zones and thereby potentially reduce oxidative damage from prolonged exposure to surface light intensities. Barbosa et al. (2003b) stated the parameters that influence the light/dark cycles are light fraction (the ratio between the light period and the cycle time) and circulation time. A short cycle time and a high light fraction result in a higher yield of biomass (Barbosa et al., 2003b).

Figure 3.3 shows the circulation time and light fraction measured in the gas-lift bioreactor at a depth of one-meter at different gas flow rates (1-16 $Lmin^{-1}$). The one-meter depth was an arbitrary value, but does represent a substantial increase (3-7 times higher) over traditional open systems. Each reported value is the average of fifteen measurements under the same conditions with the standard error displayed.

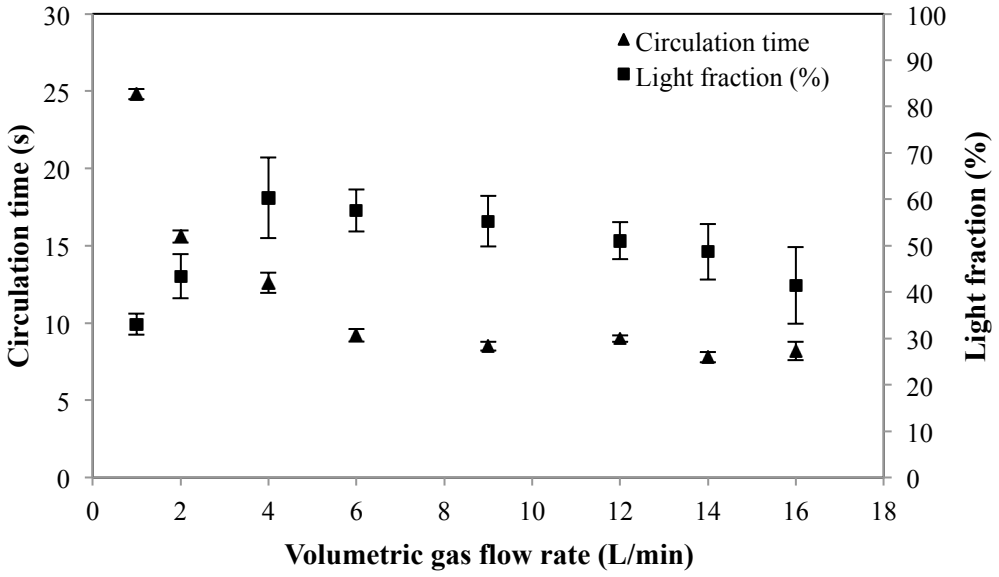


Figure 3.3: Impact of gas flow rate on circulation times and light fractions

Increasing volumetric flow rate or power input decreases the circulation time. A short circulation time is considered optimal for a photobioreactor producing microalgae, as it results in more efficient mixing due to more frequent passing through the completely mixed head zone above the riser (Sánchez Mirón et al., 2004) and reduces the length of the light/dark cycles (Barbosa et al., 2003). A small rise in circulation time for a gas flow rate higher than 9 $Lmin^{-1}$ is similar to the observation of Sánchez Mirón et al. (2004) due to enhanced gas holdup in the downcomer related to increased turbulence. Janssen et al. (2000) proposed that the optimal conditions for algae require 20% of the systems cycle to be in the dark region and that efficiency decreases significantly when the time within the dark zone is above 50% of the overall cycle time. Volumetric flow rates less than 4 $Lmin^{-1}$ and higher than 14 $Lmin^{-1}$ resulted in smaller light fraction due to respectively long cycle times and very short passes through the light zone. Within the range of 4-14 $Lmin^{-1}$ the light fraction was at the preferred interval for optimal growth (50-80%).

A mid-range volumetric gas flow rate of 9 $Lmin^{-1}$ was, therefore, selected to evaluate the feasibility of using a top-lit gas-lift bioreactor for growing microalgae.

3.3.2 Growth profile and CO_2 sequestration

A comparative study was done with respect to the growth profile of *Scenedesmus dimorphus* in the top-lit gas-lift bioreactors using air (CO_2 content of air around 0.038% v/v) and also CO_2 enriched air to give CO_2 levels of 3% and 6% (v/v). Starting with similar initial cell concentrations and the same light level, the changes in the dry weight of biomass of the cultures over three weeks of experiment are presented in Figure 3.4.

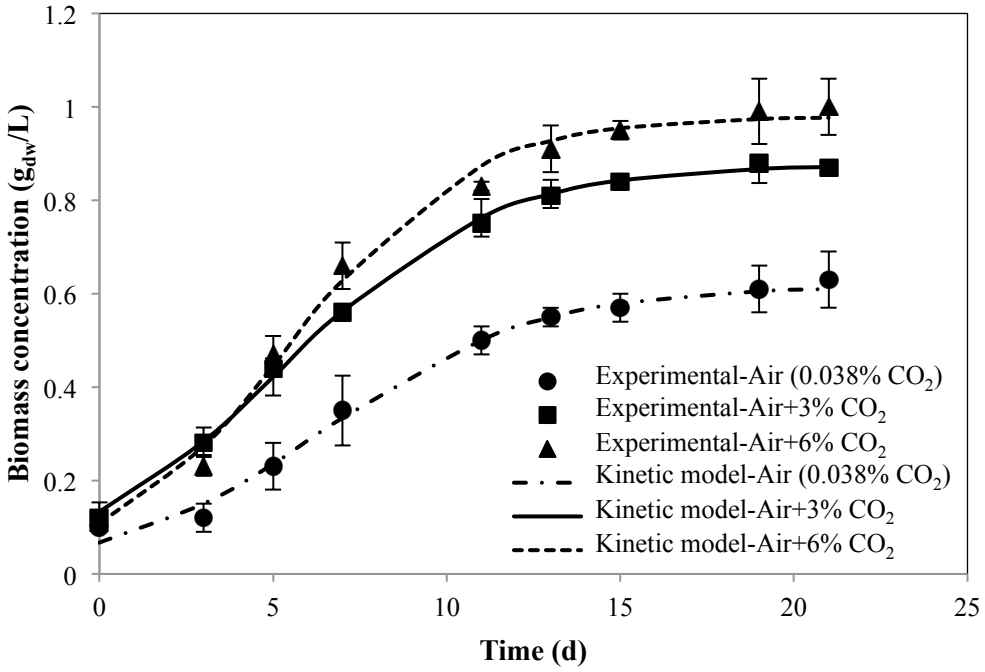


Figure 3.4: Growth profiles and kinetic model fits (Eq.5) of *Scenedesmus* sp. in the top-lit gas-lift bioreactors; Error bars show the standard error of triplicate experiments

As can be seen from Figure 3.4, enhancing the CO_2 content of the sparged gas improved the growth rates. However, the difference between growth of cultures sparged with 3%

and 6% CO_2 was not so pronounced. Increasing the CO_2 levels in the gas stream resulted in a decreased pH level of the culture (Figure 3.5), presumably from higher levels of carbonic acid. This can lead to restricted growth, despite a greater supply of carbon, due to inhibited RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) activity in the CO_2 concentrating mechanism (CCM) (Zhao and Su, 2014). It is reported that a high pH value is preferable for CO_2 absorption (Weissman et al., 1988), but optimal growth rate of most microalgal species is achieved at around pH 7 to 8 (De Godos et al., 2014). Decreased pH values in cultures sparged continuously with CO_2 also agrees with similar observations by Putt et al. (2011) due to equilibrium achieved in CO_2 input, consumption and output rates. In the absence of supplemental CO_2, pH is elevated to around 8 due to activity of the carbonic anhydrase (CA) enzyme transferring hydroxide ions outside the cell (Kumar and Das, 2012). Therefore, maintaining a good balance between growth rate and CO_2 absorption rate requires a controlled pH to achieve optimum performance.

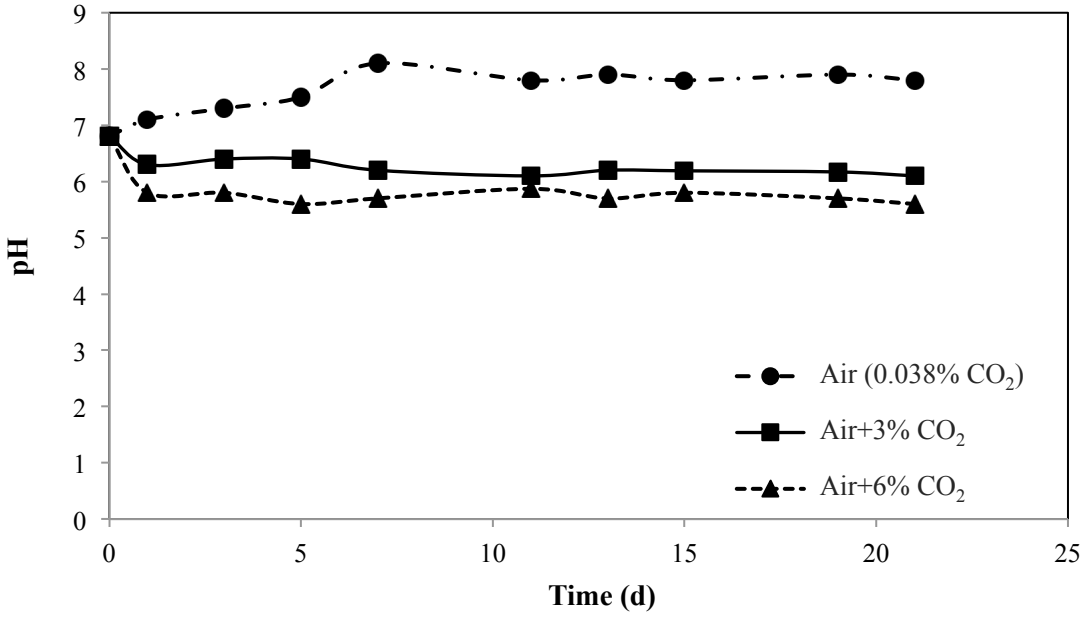


Figure 3.5: pH variations of the cultures

In addition, it could be concluded that increasing the CO_2 content of the gas sparged to the bioreactor from 3% to 6%, resulted in more loss of unused carbon dioxide to the atmosphere. Ketheesan and Nirmalakhandan (2012) had reported the same issue with a 23-L airlift-raceway bubbled with 1% and 3% CO_2 in air. Therefore, intermittent use of gas in a series of one-meter deep gas-lift bioreactors could be considered as a means of enhancing the CO_2 utilization efficiency and culture productivity by recapturing the unused CO_2 content of output gas by recycling it.

The net specific growth rate (day^{-1}), volumetric productivity ($g_{dw}L^{-1}day^{-1}$), areal productivity ($g_{dw}m^{-2}day^{-1}$) and CO_2 sequestration rate ($g_{CO2}m^{-2}day^{-1}$) are reported in Table 3.1. The bioreactor fed with 6% CO_2 had the maximum growth rate due to the presence of excess CO_2. The biomass concentration rose from 0.11 to 0.95 $g_{dw}L^{-1}$ at the exponential phase of growth.

Table 3.1: Volumetric and areal biomass productivities, CO_2 sequestration and specific growth rates at different CO_2 content in the feed gas

CO_2 content (%)	Specific growth rate (day^{-1})	Volumetric biomass productivity ($g_{dw}L^{-1}day^{-1}$)	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	CO_2 sequestration rate ($g_{CO2}m^{-2}day^{-1}$)
0.038	0.130	0.034	33.6	61.4
3	0.147	0.051	51.4	94.1
6	0.163	0.060	60.0	109.8

The volumetric productivity ($g_{dw}L^{-1}day^{-1}$) achieved by using our one-meter deep, gas-lift bioreactor with additional CO_2 is comparable with values reported for shallow outdoor raceways in batch mode (Table 3.2). However, higher productivity reported in other studies (Ketheesan and Nirmalakhandan, 2012) generally were obtained from outdoor cultivation using sunlight energy and/or with controlled-pH at a favorable level for the

tested species. They were also operated in semi-continuous mode at around a 10 cm depth, which is ideal for light penetration and photosynthetic activity. In another study using an airlift loop in a 2,200 L enclosed rectangular prism tank, Zimmerman et al. (2011) obtained 0.02 $g_{dw}L^{-1}day^{-1}$ for outdoor cultivation of *Dunaliella salina.*

Table 3.2: Reported open raceway (batch mode) volumetric and areal biomass productivities

Algal strain	Volumetric productivity ($g_{dw}L^{-1}day^{-1}$)	Areal productivity ($g_{dw}m^{-2}day^{-1}$)	Depth (cm)	CO_2 Addition	Controlled pH	Reference
Chlorella sp.	0.026	26.0	100	Yes	6.5	(Mayer et al., 1964)
Scenedesmus and *Colostrum*	0.086	38.7	45	No	No	(Al-Shayji et al., 1994)
Mixotrophic strains	0.057	10.4	18	Yes (5-6%)	No	(Chinnasamy et al., 2010)
Scenedesmus sp.	0.085	4.3	~5	Yes (1%)	No	(Ketheesan and Nirmalakhandan, 2012)
Spirulina sp.	0.060	6.0	10	Yes	9.8	(Bao et al., 2012)
Nanochloropsis sp.	0.208	10.4	5	N/A	N/A	(Chiaramonti et al., 2013)
Scenedesmus sp.	0.060	60.0	100	Yes (6%)	No	This study

From Table 3.2, it can be seen that productivity per unit area obtained in our one-meter deep bioreactor is around three-times higher than the average areal productivity reported in the literature. De Godos et al. (2014) achieved productivity of 17 $g_{dw}m^{-2}day^{-1}$ by growing *Scenedesmus* sp. at 20 cm depth, in an outdoor raceway equipped with one-meter deep sump, at a controlled pH of 8.0 and sparged with 10% CO_2 in a semi-continuous mode. Mayer et al. (1964) reported a productivity of 26 $g_{dw}m^{-2}day^{-1}$ in a one-meter deep open culture illuminated from the top and the south facing side, and continuously stirred at a controlled pH of 6.5. Whilst through substituting a paddlewheel with a propeller (axial pump) and reducing pond depth to 5 cm, Chiaramonti et al. (2013) obtained 10.4 $g_{dw}m^{-2}day^{-1}$.

Higher areal productivity achieved with the proposed configuration will lead to a smaller footprint on an industrial site and hence make it more feasible to locate the cultivation ponds closer to the off-gas source. This would reduce power requirements and installation costs associated with off-gas distribution.

Furthermore, a higher depth will result in a longer residence time for the gas in the culture medium and hence improve the potential for CO_2 uptake as well where appropriate heat transfer into the culture. The latter can play a vital role in cold climate regions in terms of being able to operate year-round by maintaining an optimum culture temperature (Laamanen et al., 2014). Also, deeper ponds sunk into the ground benefit from insulation by the surrounding ground to help prevent heat loss compared to traditional raceways with 15-35 cm depth. Moreover, under comparable levels of relative humidity, wind velocity and air temperature a smaller surface area per unit volume ration for an open bioreactor leads to the lower evaporative heat and water losses.

A fixation rate of CO_2 by microalgae is dependent on the temperature, pH and energy of photons (Zhao and Su, 2014). However, the absorption rate of CO_2 depends on residence time and interfacial area of gas-liquid contact. Bubbling CO_2 in a deeper gas-lift bioreactor not only provides vertical mixing, but also increases both contact time and interfacial area and will result in greater CO_2 mass transfer efficiency. The results of this study in general present higher daily CO_2 sequestration per unit area in a bench scale compared to various designs of open systems or photobioreactors. As reported in Table 3.1, the bioreactor sparged with 6% CO_2-enriched air achieved the highest CO_2 sequestration rate of 109.8 $g_{CO2}m^{-2}day^{-1}$. De Godos et al. (2014) achieved a 7.53 $g_{CO2}m^{-2}day^{-1}$ fixation rate by injecting flue gas with 10% CO_2 into a one-meter deep carbonation

sump in a 100 m^2, 20 cm deep open pond operated in a semi-continuous mode. Doucha et al. (2005) reported 39.2 $g_{CO2}m^{-2}day^{-1}$ in a 55 m^2 outdoor open thin-layer bioreactor when bubbling flue gas containing 6-8% CO_2. Hu et al. (1998) reported 200.4 $g_{CO2}m^{-2}day^{-1}$ in a flat-plate reactor by supplying 5% CO_2 to 11.4 L of culture with a 1 cm light path and under continuous illumination of 2,000 $\mu molm^{-2}s^{-1}$.

3.3.3 Kinetics of growth

The described logistic model (Section 3.2.5) fitted well with the experimental results obtained at different CO_2 concentrations (Figure 3.4). The simulated values of specific growth rate, initial and maximum biomass concentrations are reported in Table 3.3.

Table 3.3: Logistic model parameters with R^2 values at different CO_2 contents

CO_2 content (%)	Initial biomass concentration C_{b0} ($g_{dw}L^{-1}$)	Maximum biomass concentration C_{bmax} ($g_{dw}L^{-1}$)	Specific growth rate μ (day^{-1})	R^2
0.038	1.07	0.62	0.32	0.99
3	1.15	0.87	0.33	0.98
6	1.13	0.98	0.37	0.98

The R^2 values show a good agreement between the model and experimental data. Initial and maximum biomass concentrations (C_{b0} , C_{bmax}) estimated by the model agreed closely with the measured data for all the experimental conditions. However, the predicted specific growth rate (μ) was around 2.2-2.5 times greater than the net specific growth rate stated in Table 3.1. A similar observation was reported by Kumar and Das (2012), as their prediction was 1.8 times higher than the result obtained with a 5% CO_2 in air in a gas-lift bioreactor. This could be explained by limitations caused by nutrient deprivation and/or by self-shading inhibiting the growth rate of microalgal cells.

3.3.4 Volumetric mass transfer coefficient

The volumetric mass transfer coefficients (K_La) of O_2 in the riser and the downcomer sections calculated from the slope of Eq. 7 were 79.2 h^{-1} (R^2=0.96) and 51.1 h^{-1} (R^2=0.99), respectively. The volumetric mass transfer coefficients of CO_2 at 22°C were then calculated with Eq. 8 as 70.5 h^{-1} and 45.5 h^{-1}, respectively.

The K_La value of the riser was around 1.5 times greater than the K_La value of the downcomer due to a lower gas holdup in downcomer resulting in a longer time required to reach saturation. Similar results were observed by Kumar and Das (2012). However at a gas flow rate of 9 $Lmin^{-1}$, the high turbulent intensity and smaller sized bubbles in the riser resulted in bubbles being "dragged" into the downcomer, as Sánchez Mirón et al. (2000) pointed out, and can result in enhanced gas holdup and hence further mass transfer of CO_2 in the downcomer. This could, therefore, counteract the negative effect of shorter gas-liquid contact time due to a high bubble rise velocity in the riser.

3.3.5 Lipid content

The total lipid was extracted from the biomass as described in Section 3.2.7. The percentages of total lipid content per dry weight of biomass (% $g_{lipid}/g_{dw,biomass}$) are shown in Figure 3.6. An increasing trend in lipid content, which varied between 14-19% at day 21, with addition of CO_2 level in air was observed. Vidyashankar et al. (2013) reported a comparable lipid content of 10-20% for *Scenedesmus dimorphus* growing under 0-2% (v/v) CO_2 in air.

Compared to the culture sparged with air, those exposed to 3% and 6% CO_2 increased their lipid content by 24% and 27%, respectively. A similar observation of a lipid content increase with the higher CO_2 concentration was reported in the study of Tang et al. (2011). They found lipid content increased by 9% and 27% when bubbling with 5% and 10% CO_2, respectively. As there was a consistent hydrodynamic stress (continuous aeration, bubbles bursting, gas velocity) in all bioreactors, the increase in the lipid content can be most likely attributed to physiological stress (Eibl et al., 2014) arising from lower pH environment (Figure 3.5) caused by the enhanced CO_2 concentrations.

In terms of biofuel production (e.g., biodiesel), both biomass concentration and lipid content affect total productivity and consequently the economics of an algal plant. Therefore, lipid volumetric productions in terms of mass of lipid per unit reactor volume ($g_{lipid}L^{-1}$) for days 7, 15 and 21 are presented in Figure 3.6. It can be seen that although the decreased pH of the cultures sparged with a higher CO_2 content air resulted in diminishing returns in the growth of microalgae, as discussed in Section 3.3.2, a significant increase in lipid volumetric production occurred at the beginning of the stationary phase with higher CO_2 level. The maximum lipid volumetric production of 0.18 $g_{lipid}L^{-1}$ attained with 6% CO_2 enriched air is higher than the 0.15 $g_{lipid}L^{-1}$ obtained by Vidyashankar et al. (2013) with 5% CO_2 addition.

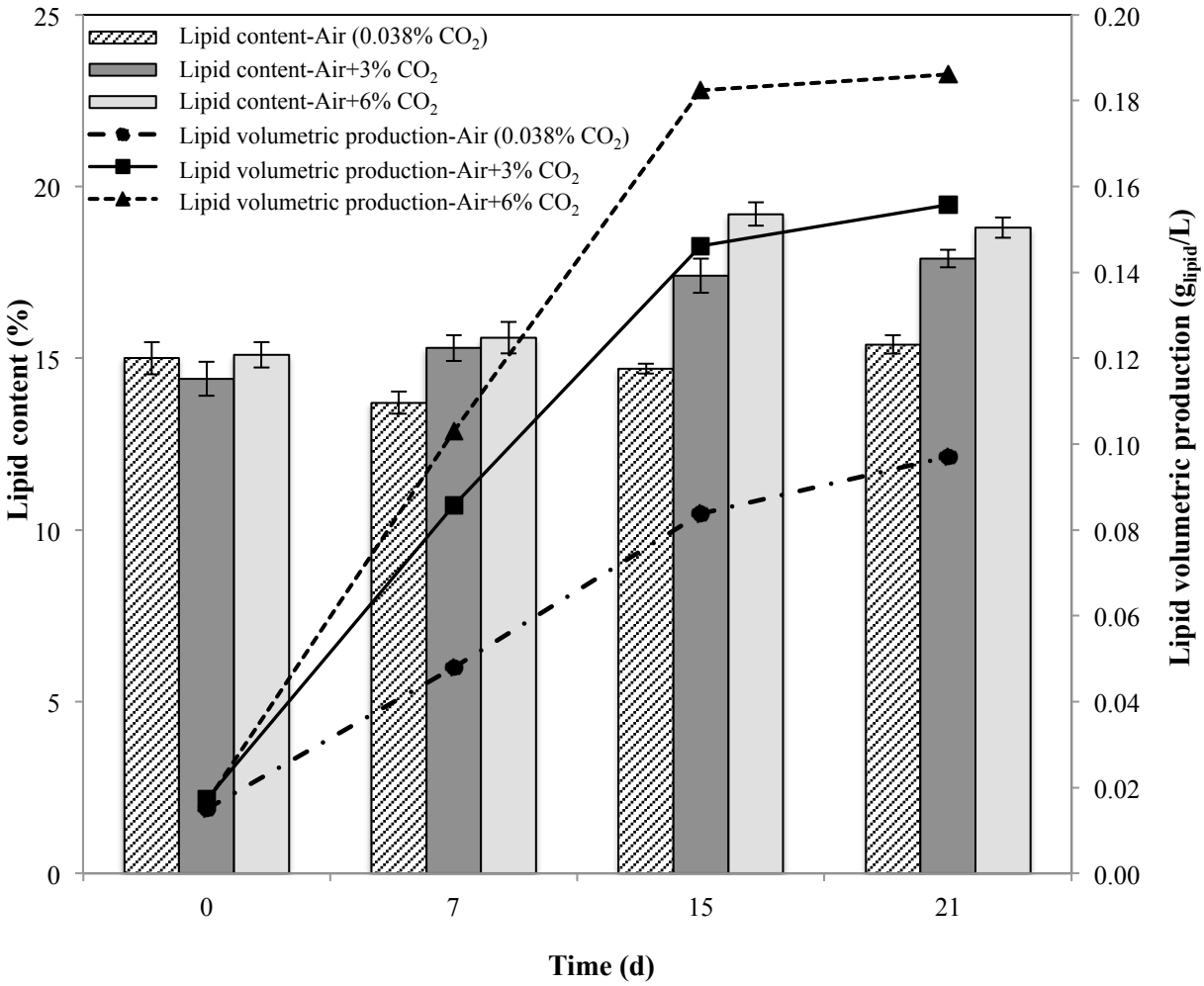


Figure 3.6: Change in microalgae lipid content (% $g_{lipid}/g_{dw,biomass}$) and lipid volumetric production (g_{lipid}/L); Lipid content is given as the mean ± standard error (triplicate experiments)

3.3.6 Power requirement

Energy consumption impacts the economic viability of algal cultivation considerably. Energetic productivity of the design proposed in this study from sparging with 6% additional CO_2 is 0.41 $g_{dw}W^{-1}day^{-1}$. This result shows a better or comparable performance to photobioreactors (Table 3.4) with similar laboratory cultivation conditions (such as aeration mixing system, artificial lighting, and temperature).

Table 3.4: Daily biomass productivity per unit power input (g_{dw}/Wday) (Modified from Ketheesan and Nirmalakhandan (2012))

Bioreactor type	Energetic productivity ($g_{dw}W^{-1}day^{-1}$)	Volume (L)	Reference
Bubble column	0.51	1.8	(Morais and Costa, 2007)
Airlift bioreactor	0.12	4	(Chiu et al., 2009)
Tubular bioreactor	0.16	0.5	(Ryu et al., 2009)
Bubble column	0.15	0.8	(Tang et al., 2011)
Airlift-raceway	0.60	23	(Ketheesan and Nirmalakhandan, 2012)
Top-lit gas-lift bioreactor	0.41	32	This study

Morais and Costa (2007) achieved an energetic productivity of 0.51 $g_{dw}W^{-1}day^{-1}$ by intermittent sparging of bubble column for 15 minutes per hour and reducing the mechanical power input significantly. A study by Ketheesan and Nirmalakhandan (2012) reported 0.6 $g_{dw}W^{-1}day^{-1}$ of biomass productivity per power input in an airlift-driven raceway with an estimated (from their reported data, (Ketheesan, 2012)) culture depth of 5 cm and a liquid velocity of 0.1 ms^{-1}.

Light energy input contributed in 68% of power requirement of the top-lit gas-lift bioreactor in this study. Thus outdoor cultivation with natural solar irradiance will impact substantially on reducing operating costs (power) and improving energetic biomass productivity.

3.4 Conclusions

The feasibility of microalgae cultivation using a one-meter deep, top-lit gas-lift open bioreactor using air with CO_2 enhanced to 6%, a level similar to that in industrial off-gas from a smelter furnace, was studied. The results indicated a comparable volumetric

productivity to traditional raceways, but around three-times greater areal productivity. This can substantially reduce the footprint of algal plant utilizing waste industrial off-gas. By being top lit only (i.e., by sun light alone in an outdoor system), this means that the costs of installing, operating and maintain supplementary lighting are avoided, as well as the tanks can be buried to provide for better insulation and cheaper installation costs.

The top-lit gas-lift bioreactor demonstrated potential, therefore, for improving the economics of microalgae production. Further studies are underway to optimize bioreactor performance by investigating the effect of a range of operational parameters on algal biomass and lipid productivities, such as CO_2 concentrations, gas flow rates and bioreactor depths.

Chapter Four: Comparative analysis of top-lit bubble column and gas-lift bioreactors for microalgae-sourced biodiesel production

Paper #3 – Original Research, Energy Conversion and Management, 130 (2016) 230-239

Nekoo Seyed Hosseini[1], Helen Shang[1], Gregory M. Ross[2], and John Ashley Scott[1,2*]

[1]Bharti School of Engineering, Laurentian University, Sudbury, ON, Canada

[2]Northern Ontario School of Medicine, Sudbury, ON, Canada

Abstract

The development of top-lit one-meter deep bioreactors operated as either a gas-lift or bubble column system using air and carbon dioxide (CO_2) enriched air was studied. The goal was high productivity cultivation of algae with elevated lipid levels suitable for conversion into biodiesel. A theoretical energy requirement analysis and a hydrodynamic model were developed to predict liquid circulation velocities in the gas-lift bioreactor, which agreed well with experimental measurements. The influence of operational parameters such as design of bioreactor, gas flow rates and CO_2 concentration on the growth and lipid volumetric production of *Scenedesmus dimorphus* was evaluated using factorial design. Whilst biomass productivity was 12% higher in the bubble column bioreactor (68.2 $g_{dw}m^{-2}day^{-1}$), maximum lipid volumetric production (0.19 $g_{Lipid}L^{-1}$) was found in a gas-lift bioreactor sparged with 6% CO_2 due to hydrodynamic and light stress.

Keywords: Microalgae, Gas-lift, Bubble column, Lipid production, Energy, Hydrodynamics

4.1 Introduction

Large-scale microalgal cultivation is typically carried out in open systems such as circular ponds with rotating arms, raceway ponds with paddlewheels, and cascade systems with baffles. They are relatively simple to construct, maintain and operate, but due to light penetration limitations have operating depths of only 15-35 cm (Demirbas, 2010). This leads to large land requirements (Vasumathi et al., 2012), which can be an issue especially if industrial off-gas to supply carbon dioxide (CO_2) (Bilanovic et al., 2012) and heat (Laamanen et al., 2014) for enhancing microalgal production of lipids for biodiesel is to be considered.

There have been only a few applications of gas-liquid contacting devices in large-scale shallow open systems with the aim of improving biomass productivity. These include placing porous stones at the bottom of ponds or diffusers at the bottom of single or multiple sumps (Bao et al., 2012; Greenwell et al., 2010), using a carbonation bubble column in conjunction with raceways (Putt et al., 2011), airlift-driven raceway design (Ketheesan and Nirmalakhandan, 2012), venturi injectors (Du et al., 2012), and a carbon dioxide supplying device fixed on the pond bottom (Su et al., 2008). However, the shallow depths lead to inefficient use of off-gas due to short gas bubble residence times which in turn impacts on biomass productivity. Raceway ponds should theoretically have production levels of 50–60 $g_{dw}m^{-2}day^{-1}$, but in practice, productivities of even 10–20 $g_{dw}m^{-2}day^{-1}$ are difficult to achieve (Shen et al., 2009).

As finding sufficient space to locate microalgae cultivation ponds close to fixed off-gas sources on an industrial site is likely to be a challenge, employing deeper ponds to

improve areal productivity could be a possible solution. An option to achieve deeper ponds, and smaller footprint and longer gas-liquid transfer times is to use vertical bubble column or gas-lift systems. This approach can improve mass transfer, provide good mixing with low stress and limit algae growth on walls. The use of bubble or gas-lift columns in deep open ponds has not, however, been widely studied and there is little comparative information between the two approaches with respect to their biomass productivities, but no consistent results have been reported. Barbosa et al. (2003b) stated that the bubble columns are more efficient for algal growth, whereas other studies showed higher biomass productivity in gas-lift columns (Kumar and Das, 2012; Oncel and Sukan, 2008; Ugwu et al., 2008).

In this study one-meter deep, top-lit bubble column and gas-lift bioreactors with regards to productivity are evaluated and compared. In particular the production of lipids that could be used as a feedstock for conversion into biodiesel (Demirbas and Fatih Demirbas, 2011). Growing conditions such as nutrients availability (Cakmak et al., 2012), pH (Eibl et al., 2014), temperature (Xin et al., 2011), light exposure (Choi et al., 2015), CO_2 content of industrial off-gases (Xia et al., 2013), and hydrodynamic conditions (Song et al., 2014) are known as triggers of lipid synthesis by microalgal cells. Therefore, differences in mixing patterns of the bubble and gas-lift columns, as well as lighting and hydrodynamic conditions are examined in terms of not just algal biomass productivity, but also lipid productivity.

4.2 Material and Methods

4.2.1 Microalgae selection and growth medium

The green microalgae *Scenedesmus dimorphus* was used in this work. It was obtained from the University of Texas, Austin collection (1,237 UTEX collection) and inoculums grown in freshwater Bold's Basal growth medium (Andersen, 2005) at 25°C.

A pre-culture was then produced in covered 180 L glass tanks (120 x 30 x 50 cm) under fluorescent light of approximately 60 $\mu molm^{-2}s^{-1}$ on a 12 hour light/dark photoperiod. The temperature was 22±2°C, and they were continuously agitated with bubbling air and supplied with Bold's Basal growth medium every three weeks.

4.2.2 The bioreactor set-up

The bioreactors used were a bubble column and a concentric draft-tube gas-lift column sparged in the draft tube with an internal diameter (D_i) of 0.13 m and height of 0.8 m (Figure 4.1). They were made from 5 mm thick, transparent plexiglas with a diameter (D_o) of 0.2 m. The columns had side ports at 0.05 m and 0.5 m from the base for taking samples. The ratio of cross sectional area of riser to downcomer was 0.73 for the gas-lift reactor and the draft tube was located 0.05 m from the bottom. The working volume was 0.03 m^3 at 1 m depth. Air mixed with carbon dioxide to achieve a 6% CO_2 mix content was sparged through a 0.10 m diameter ceramic sparger with mean pore size of 15 μm (Refractron Technologies Corp., NY, USA). The flow rate was controlled by using rotameters (Omega Engineering Ltd., QC, Canada).

The outside of the bioreactors were covered with a layer of black sheet on top of a white sheet to block light entrance from the side. Light energy for the bioreactors was provided only from the top of each column to simulate buried open systems on an industrial site, which is top lit by sunlight. A 90 watt circular grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) was used with a photosynthetic active radiation at the surface of approximately 1,000 $\mu molm^{-2}s^{-1}$. A 12 hours light/dark photoperiod was used. Make up water was supplied every day to compensate for evaporative loss and experiments were carried out at 22±2°C.

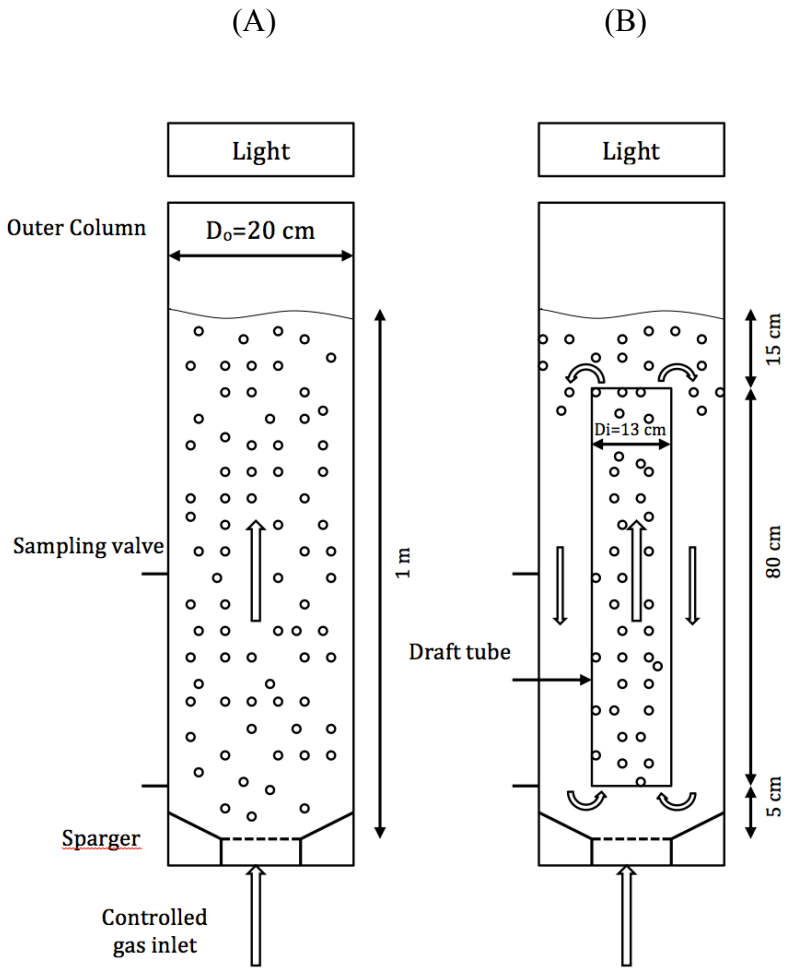


Figure 4.1: Schematic diagram of top-lit (A) bubble column and (B) gas–lift bioreactors

4.2.3 Hydrodynamics characterization

In this section, expressions for estimating the energy requirements for the top-lit bioreactors are developed from experimental and theoretical considerations.

4.2.3.1 Gas hold-up

The overall gas hold-up (ε) was calculated by measuring the increase in water column height upon aeration at specific gas flow rate:

$$\varepsilon = \frac{h_D - h_L}{h_D} \quad (1)$$

where h_D is the aerated height and h_L is the unaerated height.

4.2.3.2 Liquid circulation velocity

Average liquid circulation velocities (V_L) in the gas-lift bioreactors were calculated by dividing the length of the circulation loop (L_c) with the circulation time (t_c) at gas volumetric flow rates in the range of 4-14 $Lmin^{-1}$. Considering exponential decay of light intensity with depth, photosynthetic active radiation of 50 $\mu molm^{-2}s^{-1}$ is considered as transition from the light zone to the dark zone (Barbosa et al., 2003b). Janssen et al. (2000) found that when 50% to 80% of the circulation time was in light zone, growth would be at an optimum rate. The volumetric flow rates in this study resulted in light fractions in the acceptable interval of 50%-80% (Seyed Hosseini et al., 2015). The circulation time (t_c) was measured by timing one cycle of 5 mm diameter tracer beads with the same density as water (Engineering Laboratories, NJ, USA).

4.2.4 Energy balance

An overall energy balance for the gas-lift bioreactor can be determined based on the model described by (Chisti, 1989):

$$W_i = W_{Rr} + W_{Dd} + W_{Fr} + W_{Fd} + W_B \quad (2)$$

where W_i is the power input due to the isothermal expansion of gas, W_{Rr} power loss due to wakes behind the bubbles in the riser, W_{Dd} power loss due to stagnant gas in the downcomer, W_{Fr} and W_{Fd} power losses due to friction in the riser and downcomer, and W_B power loss due to fluid turn-around at the bottom of the bioreactor.

4.2.4.1 Power input due to isothermal expansion of gas

Power input due to isothermal expansion of gas as it rises in the gas-lift bioreactor was calculated from (Sánchez Mirón et al., 2000):

$$W_i = \frac{\rho_l g U_{Gr} A_t h_L}{1+\frac{A_d}{A_r}} \quad (3)$$

where ρ_L is the culture density which was assumed to be the density of water, g gravitational acceleration, U_{Gr} the superficial gas velocity in the riser, A_t is the total cross-sectional area, A_r and A_d the cross-sectional areas of riser and downcomer, respectively.

4.2.4.2 Power loss due to wakes behind the bubbles in the riser (W_{Rr})

Power loss due to the wakes behind the bubbles was calculated based on rate of pressure energy loss of the gas and liquid in the riser, and rate of potential energy loss of the liquid

with an assumption of uniform gas hold-up in the riser (ε_r) (Ketheesan and Nirmalakhandan, 2011):

$$W_{Rr} = \rho_l g h_r U_{Gr} A_r + \rho_l g h_r U_{lr} A_r - \frac{\rho_l g h_r U_{lr} A_r}{(1-\varepsilon_r)} \quad (4)$$

As the gas-hold up in the riser is negligible, it can be assumed that the two right hand terms of Eq. 4 cancel out and, therefore, the equation for power loss can be reduced to:

$$W_{Rr} = \rho_L g h_r U_{Gr} A_r \quad (5)$$

where h_r is the height of riser and U_{Gr} the superficial gas velocity in the riser.

4.2.4.3 Power loss due to stagnant gas in the downcomer (W_{Dd})

The energy balance over the downcomer included power dissipation due to the drag of gas on liquid, rate of pressure energy gain and rate of potential energy loss (Chisti, 1989):

$$0 = W_{Dd} + \rho_L g h_D (1 - \varepsilon_d) U_{ld} A_d - \rho_L g h_D U_{ld} A_d \quad (6)$$

and can be reduced and rearranged to:

$$W_{Dd} = \rho_L g h_D \varepsilon_d U_{ld} A_d \quad (7)$$

where U_{ld} is the superficial liquid velocity in the downcomer, and ε_d the gas hold-up in the downcomer, which can be found using ε as the overall gas hold-up from:

$$\varepsilon_d = \frac{\varepsilon(A_r + A_d) - \varepsilon_r A_r}{A_d} \quad (8)$$

The gas hold-up in the riser, ε_r, can be calculated from:

$$\varepsilon_{r=}\frac{U_{Gr}}{U_b} \quad (9)$$

where U_b is the mean bubble rise velocity, which can be estimated by the model proposed by Talaia (2007) that assumes negligible bubbles exist in the downcomer:

$$U_b = 1.5\left(\frac{g d_B(\rho_L-\rho_G)}{\rho_L}\right)^{1/2} \quad (10)$$

where ρ_G is density of mixture of air and CO_2 at 20°C and d_B, the mean bubble diameter. d_B, can be assessed by modifying the following correlation proposed by Cerri et al. (2010) based on Higbie's penetration theory:

$$d_B = \left[\frac{6D_L}{\pi(1-\varepsilon)\psi^2}\right]^{2/5}\left[\frac{g\Delta\rho}{\rho_L}\right]^{1/5} \quad (11)$$

where D_L is oxygen diffusivity in water at 20°C and ψ the relationship between gas transfer and hold-up. ψ can be calculated from experimentally obtained values of the volumetric mass transfer coefficient (k_La) and gas hold-up (ε) (Chisti, 1989):

$$\psi = \frac{k_La(1-\varepsilon)}{6\varepsilon} \quad (12)$$

The k_La of oxygen was measured by a dynamic gassing-in and gassing-out method (Sánchez Mirón et al., 2000), which is based on penetration theory. The measured oxygen absorption rate was obtained from:

$$\frac{dC}{dt} = K_La(C^* - C) \quad (13)$$

where C is the dissolved oxygen concentration at time t and C^* the dissolved oxygen saturation concentration.

4.2.4.4 Power loss due to friction (W_{Fr}, W_{Fd})

Power dissipation due to friction over the length of the riser (h_r) and downcomer (h_d) was calculated as follows (Munson et al., 1995):

$$W_{F(r,d)} = f\rho_L U_{l(r,d)} h_{(r,d)} V_{l(r,d)}^2 A_{(r,d)} / 2d_{H(r,d)} \quad (14)$$

where *f* is the Darcy friction factor at the corresponding Reynolds number, $V_{(r,d)}$ is the actual liquid velocity in the riser and downcomer and d_H is the hydraulic diameter of the riser and downcomer.

4.2.4.5 Power loss due to fluid turn-around at the bottom of bioreactor (W_B)

The power loss due to fluid turn-around at the top of the bioreactor was considered negligible compared to the constricted bottom section. The energy loss at the bottom of the gas-lift due to fluid turn-around can be then calculated (Chisti, 1989):

$$W_B = \frac{1}{2}\rho_L U_{ld}^3 A_d K_B \frac{1}{(1-\varepsilon_d)^2} \quad (15)$$

where K_B is the friction loss coefficient for the bottom connecting section and can be correlated with the free area between the downcomer and riser, A_b, in a range of 0.2 to 1.8 for A_d/A_b (Molina et al., 1999):

$$K_B = 11.402 \left[\frac{A_d}{A_b}\right]^{0.789} \quad (16)$$

By applying the overall energy balance over the gas-lift bioreactor (Eq. 2), the predicted liquid circulation velocities were estimated and compared with experimental measured velocities over a range of volumetric gas flow rates.

4.2.5 Design of experiments and statistical analysis

The experimental design employed a two-level, three-factor factorial design to evaluate the influence of bubble column (BC) and gas-lift (GL) bioreactors on biomass concentration and lipid volumetric production. Three independent variables of gas flow rates (4-14 $Lmin^{-1}$), CO_2 concentration (0-6%) and bioreactor design were tested. Table 4.1 shows the levels of the coded factors. The experiment consisted of four blocks of eight bioreactors. Blocking was applied due to the limitation of experimental set-up to run at the same time. Two center points were considered for each block and corner points were in triplicate.

Table 4.1: Levels and actual values of the factors tested

Code	Factor	Low level (-1)	High level (+1)
X_1	Gas flow rate (L/min)	4	14
X_2	CO_2 concentration (%v/v)	0.038	6
X_3	Design of Bioreactor	GL	BC

The response functions of interest were specific growth rate (μ), areal biomass productivity (P_a) and volumetric lipid production (P_L). These functions were approximated by a second-degree polynomial to identify the significant main factors and interaction effects by using the method of least squares, as shown by Eq. (17):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j \quad (17)$$

where Y stands for the response, β_0 the independent coefficient, β_i the linear coefficient associated to each factor X_i and β_{ij} $(i \neq j)$ the quadratic coefficients associated with the interaction of X_i and X_j.

4.2.6 Growth determination

Microalgal growth was tracked by measuring optical density using a spectrophotometer (UV-1700 UV-VIS, Shimadzu, Tokyo, Japan) at 550 nm wavelength. Dry weight of the biomass (C_b) was determined by vacuum filtration of 100 ml of algal culture through glass microfiber filter paper (Grade 151, Ahlstrom Filtration LLC, PA, USA) followed by oven-drying over night at 60°C.

Specific growth rate (μ) was calculated from the initial (C_{bi}) and final (C_{bf}) dry biomass concentration over the logarithmic growth phase (Δt) as follows (Andersen, 2005):

$$\mu = \frac{\ln\left(\frac{C_{bf}}{C_{bi}}\right)}{\Delta t} \tag{18}$$

Volumetric biomass productivity (P_v) was calculated from the following:

$$P_v = \frac{C_{bf} - C_{bi}}{\Delta t} \tag{19}$$

Areal biomass productivity (P_a) was then determined using the total volume (V_t) and occupied surface area (A_t) of the bioreactor:

$$P_a = \frac{1000 P_v V_t}{A_t} \tag{20}$$

4.2.7 Lipid analysis

The lipid content of the microalgae was extracted through a modification of the method described by Folch et al. (1957). Algal biomass from each bioreactor was harvested by centrifugation, frozen at -80°C and subsequently freeze-dried at -50°C under vacuum for

24 h. Freeze-dried biomass was then mixed with 1.2 mL of chloroform:methanol (2:1 v/v) in a centrifuge tube and sonicated using a Sonic Dismembrator Model 500 (Fisher Scientific, Ottawa, Canada) for 30 minutes. The samples were centrifuged using an Allegra X-15R Centrifuge (Beckman, Palo Alto, CA) and the supernatant was removed into a weighed tube. Extraction was repeated in triplicate with the collected solvent layers being combined. The solvent was then evaporated in a vacuum drier and the mass of the remaining lipid determined gravimetrically.

4.3 Results and discussion

4.3.1 Hydrodynamics measurements

Parameters required to estimate the energy dissipation in the top-lit bioreactors are calculated by the methods described in section 4.2.3.

4.3.1.1 Mean bubble size

The volumetric mass transfer coefficient (k_La) of oxygen was calculated from Eq. (13) for various superficial gas velocities in order to estimate the mean bubble size (d_B). The k_La data obtained is given in Figure 4.2A and expressed in terms of the semi-empirical model by Sánchez Mirón et al. (2000):

For the gas-lift bioreactor:

$$k_La = \frac{0.049}{U_G^{-0.219}-1} \quad (21)$$

For the bubble column bioreactors:

$$k_L a = \frac{0.102}{U_G^{-0.301} - 1} \quad (22)$$

The results of mean bubble diameter by the proposed modified correlation (Eq. 11) presented in Figure 4.2B are in agreement with the constraints of the model proposed by Talaia (2007). It can be observed that an increase in superficial gas velocity resulted in a slight increase of the bubble diameter in a range of 2.0 to 3.9 mm. A similar observation of the weak influence of superficial gas velocity on mean bubble size was also reported by Tung et al. (1998). Both $k_L a$ and d_B values are higher in the bubble column compared to the gas-lift bioreactor. This was due to bubble agglomeration and coalescence as a consequence of longer residence times and a lack of liquid circulation. Cerri et al. (2010) reported similar trends in the bubble column and gas-lift bioreactors.

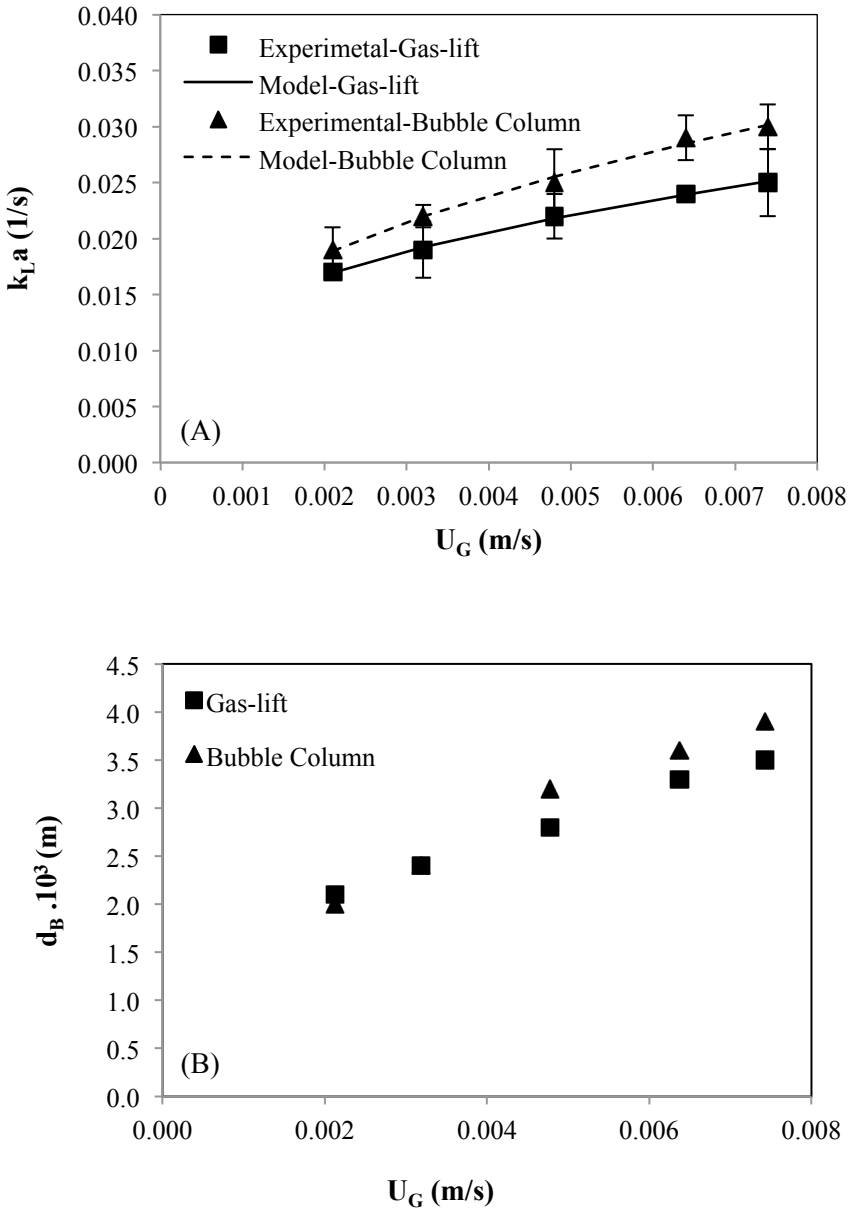


Figure 4.2: (A) Measured volumetric mass transfer coefficient (k_La). Error bars indicate the standard error of triplicate observations; (B) Mean bubble diameter (d_B) as a function of superficial gas velocity (U_G)

4.3.1.2 Gas hold-up

Gas hold-up needs to be measured in order to predict power losses in the riser and downcomer of the gas-lift bioreactor. The gas hold-up data for the riser and downcomer are presented in Figure 4.3A.

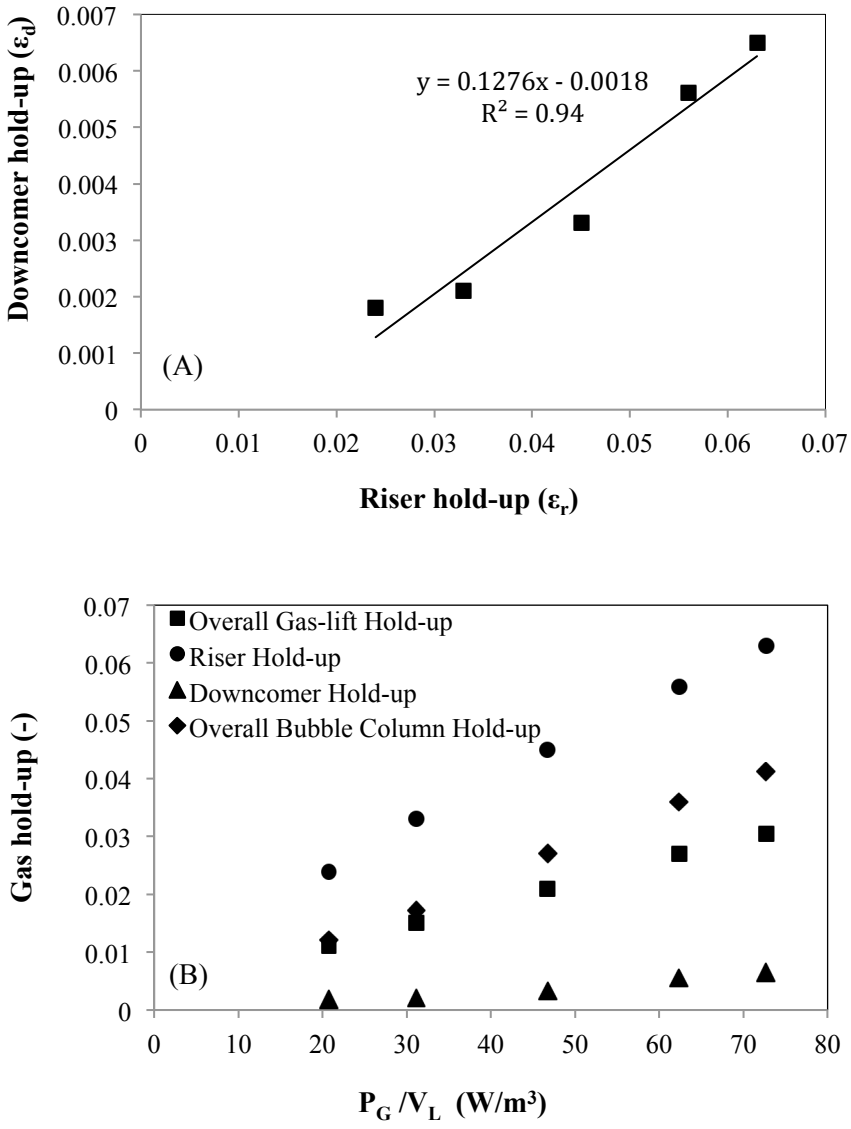


Figure 4.3: (A) Relationship between riser and downcomer gas hold-ups in the gas-lift bioreactor; (B) Comparison of gas hold-up values for various values of specific power input

The relationship between the riser and downcomer gas hold-ups shows a good fit with the linear correlation proposed by Contreras et al. (1998) with nonzero intercepts. Experimental observations are also in agreement with the assumption of a gas-free downcomer at lower gas flow rates until the gas hold-up has been built up in the riser.

The overall gas hold-up in the bubble column and gas-lift bioreactors as well as the riser and downcomer gas hold-up values for various specific power inputs (Wm^{-3}) are compared in Figure 4.3B. P_G is the power input due to aeration and V_L is bioreactor operating volume. At a specific power input, gas hold-up in the downcomer is significantly smaller than the riser. Increasing the power input resulted in an increase of the bubble column and gas-lift overall hold-ups and riser gas hold-up. However, only a small rise in the downcomer hold-up was observed. This is not consistent with the studies of Rengel et al. (2012) and Sánchez Mirón et al. (2000) and can be explained by variation in bubble sizes due to the spargers used in our work and that of others. The sparger pores were 15 μm in this work, whereas they were 1 to 2 mm in the other reported studies. The gas hold-up of the bubble column is higher than that of gas-lift due to the lower bubble rise velocity and bubble accumulation.

4.3.1.3 Liquid circulation velocity

The average liquid circulation velocities were calculated by dividing the length of the circulation loop (L_c) by the measured circulation time (t_c) at gas volumetric flow rates in the range of 4-14 $Lmin^{-1}$. The measured experimental velocities are compared in Figure 4.4 with those predicted from the theoretical energy analysis developed in Section 4.2.4. The predicted and measured velocities agree within ±20% for the gas-lift bioreactor.

However, an increase in gas flow rate affects the predicted liquid velocities significantly as energy dissipation due to wakes behind the bubbles in the riser represent 80% of the total energy losses. Ketheesan and Nirmalakhandan (2011) also reported a similar observation in a 23 L airlift driven system.

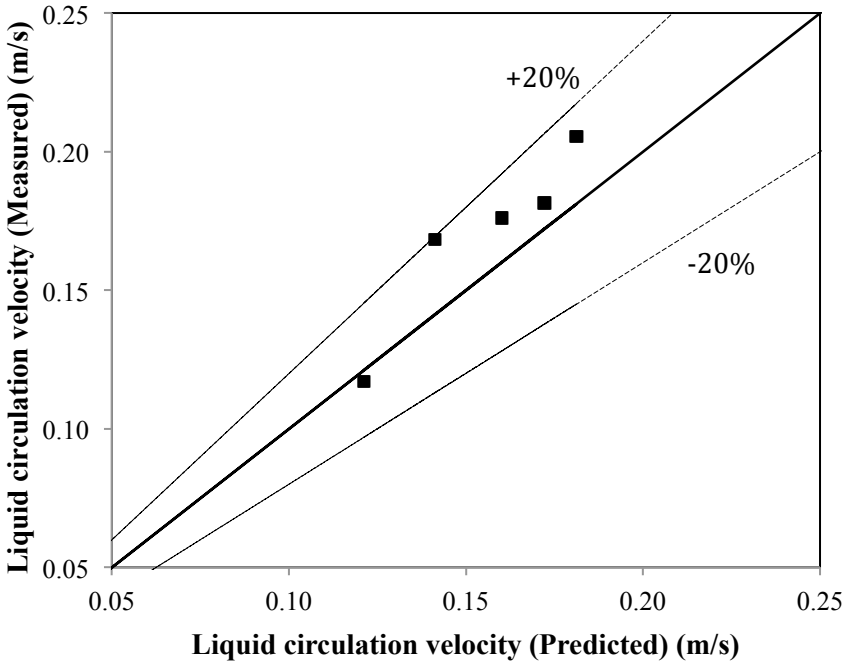


Figure 4.4: Measured vs. predicted liquid circulation velocities at different volumetric gas flow rates (4-14 L/min)

4.3.2 Statistical analysis

In order to evaluate the influence of the operational factors of gas flow rate (X_1), CO_2 concentration (X_2) and the reactor design (X_3) on areal biomass productivity and lipid accumulation, classical analysis was implemented on the values of three responses: specific growth rate (μ), areal biomass productivity (P_a) and volumetric lipid production

(P_L). Table 4.2 presents the experimental configurations suggested by full factorial design for each treatment and the obtained values for μ, P_a and P_L.

Table 4.2: Experimental design and results using the full factorial method

Run	Factors Assignment			Responses		
	X_1	X_2	X_3	Specific growth rate (day^{-1})	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	Lipid volumetric production ($g_{Lipid}L^{-1}$)
1	+1	-1	+1	0.096	28.9	0.063
2	-1	+1	-1	0.134	60.9	0.173
3	+1	-1	+1	0.087	27.8	0.078
4	-1	+1	-1	0.141	61.0	0.196
5	0	0	-1	0.096	32.4	0.150
6	+1	-1	+1	0.079	26.7	0.096
7	-1	+1	-1	0.137	60.8	0.206
8	0	0	+1	0.104	43.8	0.120
9	+1	+1	-1	0.088	36.7	0.155
10	+1	+1	-1	0.080	37.3	0.193
11	-1	-1	+1	0.103	36.4	0.069
12	0	0	+1	0.116	51.2	0.137
13	-1	-1	+1	0.091	32.7	0.099
14	-1	-1	+1	0.096	34.5	0.097
15	+1	+1	-1	0.085	38.0	0.177
16	0	0	-1	0.099	39.6	0.139
17	+1	-1	-1	0.055	18.2	0.070
18	+1	-1	-1	0.096	30.9	0.078
19	-1	+1	+1	0.147	66.4	0.147
20	-1	+1	+1	0.139	71.8	0.169
21	0	0	+1	0.119	50.7	0.100
22	0	0	-1	0.088	48.5	0.159
23	+1	-1	-1	0.075	24.3	0.056
24	-1	+1	+1	0.129	66.5	0.187
25	+1	+1	+1	0.096	43.8	0.121
26	-1	-1	-1	0.126	44.4	0.089
27	0	0	-1	0.103	41.1	0.167
28	+1	+1	+1	0.093	45.4	0.149
29	0	0	+1	0.105	48.7	0.158
30	-1	-1	-1	0.079	26.7	0.078
31	-1	-1	-1	0.106	36.4	0.056
32	+1	+1	+1	0.097	51.5	0.155

By employing quadratic least squares (QLS), the terms of the model were acquired, and the root mean square error was then calculated for each response. Regarding specific growth rate, gas flow rate (X_1) and CO_2 concentration (X_2), and the interaction of these two factors (X_1X_2) were significant at a confidence level of 95% (p-value < 0.05). The factor X_1 (p=0.005) had a negative effect on the specific growth rate, while both X_2 (p=0.000) and the interaction of X_1X_2 (p=0.004) had positive effects.

When considering the areal biomass productivity response, two factors and two interactions were found significant: X_2 (p=0.000) exerted a positive effect over the response, while X_1 (p=0.002) and both interactions of X_1X_2 (p=0.001) and X_2X_3 (p=0.051) demonstrated the opposite effect. The R^2 corresponding to the specific growth rate and areal biomass productivity models were 80.12% and 90.56%, representing a good fit of the responses. The reactor type (X_3) did not, however, have significant effect on biomass productivity (p=0.772).

By applying classical analysis for lipid volumetric production, the CO_2 content (X_2) and the interactions of CO_2 content and the reactor design (X_2X_3) were found significant with p-values of 0.000 and 0.015, respectively. In order to satisfy the model hierarchy requirement and avoiding the lack of fit, the first order of X_1 and X_3 factors were included in the model. Both X_1 and X_3 generated a negative effect over the lipid production, while X_2 and interaction of X_2X_3 exerted the contrary effect. The value of R^2 corresponding to the model was 85.13%, showing that 85% of system variability could be accurately predicted by the model. The lack of fit was not significant in any of the three reduced models, confirming the high reliability of the models for predictions and associated

probability values of all responses were $<1x10^{-6}$. Equations (23-27), are mathematical presentation of the three responses:

$$\mu = 0.108 - 0.002\ X_1 + 0.008\ X_2 - 0.005\ X_1X_2 \quad (23)$$

For gas-lift bioreactor:

$$P_a = 38.485 - 0.905\ X_1 + 5.176\ X_2 - 0.224\ X_1X_2 \quad (24)$$

$$P_L = 0.0909 - 0.0015\ X_1 + 0.0187\ X_2 \quad (25)$$

For bubble column bioreactors:

$$P_a = 40.37 - 0.905\ X_1 + 6.413\ X_2 - 0.224\ X_1X_2 \quad (26)$$

$$P_L = 0.0992 - 0.0015\ X_1 + 0.0118\ X_2 \quad (27)$$

Only significant factors have been included in the models and Table 4.3 summarizes the results obtained by applying analysis of variance (ANOVA) to the experimental data.

From analyzing the mathematical models, it can be confirmed that both lipid and biomass production are favored by increasing the CO_2 content of the feed gas and at lower volumetric gas flow rates. This analysis also confirmed that lipid production yield is favored by using a gas-lift bioreactor compared to a bubble column.

Table 4.3: Analysis of variance showing the regression model reduced to significant terms

Factor	μ (day^{-1})		P_a ($g_{dw}m^{-2}day^{-1}$)		P_L ($g_{Lipid}L^{-1}$)	
	Regression coefficient	*p*-value	Regression coefficient	*p*-value	Regression coefficient	*p*-value
Regression coefficients including all factors and their second-order interactions						
Constant	**0.1084**	**0.000**	**39.43**	**0.000**	**0.0890**	**0.000**
X_1	**-0.0020**	**0.005**	**-0.905**	**0.002**	-0.0008	0.489
X_2	**0.0082**	**0.000**	**5.794**	**0.000**	**0.0173**	**0.000**
X_3 (GL)	0.0040	0.431	0.610	0.772	-0.0069	0.440
X_1X_2	**-0.0005**	**0.004**	**-0.224**	**0.001**	-0.0002	0.400
X_1X_3 (GL)	-0.0007	0.133	-0.173	0.364	0.0003	0.699
X_2X_3 (GL)	-0.0003	0.644	**-0.618**	**0.058**	**0.0034**	**0.015**
Regression coefficients including significant factors for reduced model						
Constant	**0.1084**	**0.000**	**39.43**	**0.000**	**0.0951**	**0.000**
X_1	**-0.0020**	**0.006**	**-0.905**	**0.002**	**-0.0015**	**0.070**
X_2	**0.0082**	**0.000**	**5.794**	**0.000**	**0.0153**	**0.000**
X_3 (GL)	N/A	N/A	-0.943	0.451	-0.0041	0.426
X_1X_2	**-0.0005**	**0.005**	**-0.224**	**0.001**	N/A	N/A
X_1X_3	N/A	N/A	N/A	N/A	N/A	N/A
X_2X_3 (GL)	N/A	N/A	**-0.618**	**0.057**	**0.0034**	**0.013**

- Bold values represent statistically significant results

4.3.3 Growth rate and lipid yield

A study of *S. dimorphus* growth rate and lipid production was carried out in both one-meter deep top-lit bubble columns and gas-lift bioreactors sparged with air (CO_2 content of air around 0.038%) and 6% CO_2-enriched air. Gas flow rates (4-14 $Lmin^{-1}$) were chosen to give a preferred light fraction allowance of 50-80% (Seyed Hosseini et al., 2015; Janssen et al., 2000). Experimental runs had a three-week duration and were conducted in triplicate (Figure 4.5).

In all cases, the stationary phase in bioreactors fed with CO_2 enriched air occurred later and for the same conditions, the bubble column gave a slightly better growth rate

compared to the gas-lift. The latter was presumably due to a lower bubble residence time in the gas-lift bioreactor. Therefore, the difference was more pronounced when bubbling with higher CO_2 concentrations due to greater CO_2 transfer rates into the culture media. Kumar and Das (2012) reported similar observations with sparging bubble column and airlift bioreactors with air, and 5% and 10% CO_2-enriched air.

Lower gas rising velocities led to higher biomass concentration as the gas-liquid transfer time was increased. The maximum dry weight of biomass (1.01 $g_{dw}L^{-1}$) was achieved when 6% CO_2 (v/v) was bubbled in the bubble column bioreactor at a flow rate of 4 $Lmin^{-1}$. Conversely, increasing the gas flow rate resulted in a negative effect on biomass concentration, which was likely due to cell damage caused by bubble formation at the sparger and small bubble bursting on the surface of the microalgae culture (Barbosa et al., 2003a). Lebeau and Robert (2003) and Mirón et al. (2003) reported similar trends due to aeration-induced hydrodynamic stress. It has been found that higher aeration rates up to a critical level improved the yield of the reactor, but increasing the rate more than that optimum level, a value that is strain dependent (Khoo et al., 2016; Barbosa et al., 2003a), resulted in shear stress and decreased growth rate.

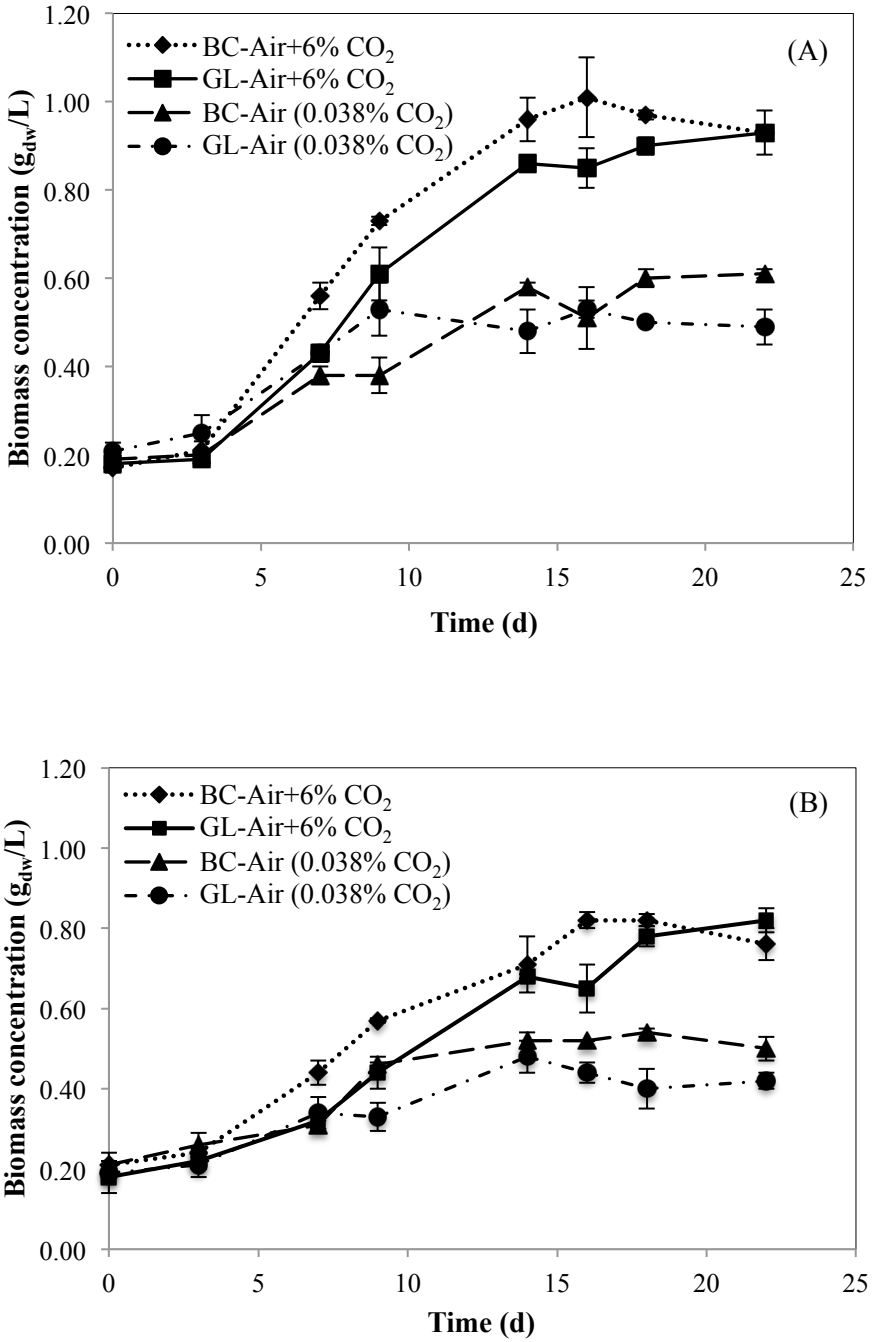


Figure 4.5: Growth profiles of *Scenedesmus* sp. in top-lit bubble column (BC) and gas-lift bioreactors (GL) sparged with either air or 6% CO_2-enriched air (Fig 4.5A at gas flow rates of 4 L/min and Fig 4.5B at 14 L/min). Error bars indicate the standard error of triplicate observations.

The areal productivities obtained ($g_{dw}m^{-2}day^{-1}$) are reported in Table 4.4. The highest areal productivity (68.2 $g_{dw}m^{-2}day^{-1}$) was achieved in a bubble column and was 12% higher than that obtained in the gas-lift bioreactor under the same operational conditions due to the higher residence time of gas in the culture medium. Compared to an average of previously published areal productivities of 20 $g_{dw}m^{-2}day^{-1}$ in open ponds (Seyed Hosseini et al., 2015), that of the deep top-lit bubble column was increased by more than three times.

Table 4.4: Areal biomass productivities ($g_{dw}m^{-2}day^{-1}$) at different CO_2 levels in the feed gas and gas flow rates in bubble column and gas-lift bioreactors

Bubble column				Gas-lift bioreactor			
Air		6% CO_2		Air		6% CO_2	
4 L/min	14 L/min	4 L/min	14 L/min	4 L/min	14 L/min	4 L/min	14 L/min
34.5	27.8	68.2	46.9	35.8	24.5	60.9	37.3

As lipid for biodiesel is the required final product, lipid content (% $g_{lipid}/g_{dw,biomass}$) and specifically lipid volumetric production (P_L) is a key factor. Figure 4.6 presents, therefore, total lipid content (bar chart) and volumetric lipid production (line chart) during the cultivation phase.

Enhanced lipid content due to physiological stress (Eibl et al., 2014) including higher CO_2 concentration and consequently lower pH of culture has been demonstrated previously (Seyed Hosseini et al., 2015; Xia et al., 2013). The pH profiles over the course of the experimental runs were similar in both the bubble column and gas-lift bioreactors. The initial pH was 6.9 for bioreactors, but dropped to 5.7 in those bubbled with CO_2-enriched air (Seyed Hosseini et al., 2015). It can be seen from Figure 4.6 that cultures

from the gas-lift bioreactors had higher increased algal lipid content compared to those from bubble columns. At gas flow rates of 4 and 14 $Lmin^{-1}$ and sparging with 6% CO_2, the lipid content of the algae in the gas-lift bioreactors increased by 39% and 48% respectively, compared to 25% and 30% in the bubble columns. As there were comparable cultivation conditions in terms of nutrient levels, pH, temperature, light intensity, and gas flow rate, this is presumably due to differences in mixing patterns and dark/light regimes. That is, as a consequence of the so-called flashing light effect provided in the gas-lift bioreactors by continual passes through light/dark zones.

Napolitano (1994) reported light-induced changes in algal lipid levels and a 58% increase in lipid content of *I. Galbana* was reported under blue intermittent light (Yoshioka et al., 2012). Choi et al. (2015) did report an increase in total fatty acids production due to light stress (flashing time of 5 times per min) during cultivation of an *A.Obliquus* species. However, Kim et al. (2014) found no significant changes in biomass and lipid productivity with multistage flashing illumination over 72 h of cultivation of *C. reinhardtii.*

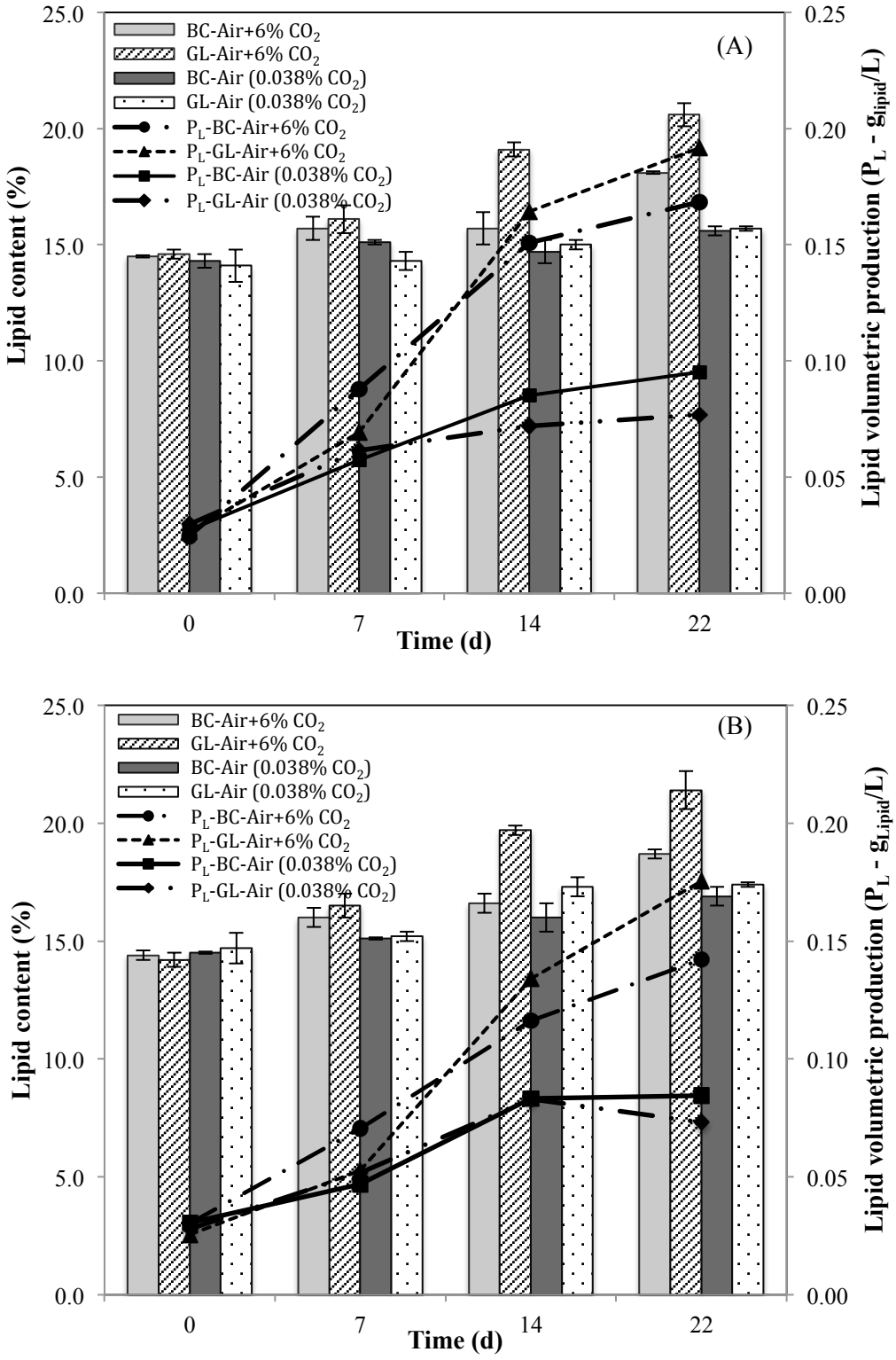


Figure 4.6: Lipid content (Bar chart) and volumetric production (Line chart) in top-lit bubble column (BC) and gas-lift bioreactors (GL) under sparging with either air or 6% CO_2-enriched air (Fig 4.6A at gas flow rates of 4 L/min and Fig 4.6B at 14 L/min). Error bars indicate the standard error of triplicate observations.

By comparing the total lipid content of microalgal cultures in relation to the gas-flow rate, it can be also concluded that the higher the superficial gas velocity, the greater the lipid content. Song et al. (2014) reported a 25% increase in the lipid content of *P. tricornutum* at an elevated gas to liquid ratio. This was attributed to enhanced turbulence, shear stress and frequency of light exposure. Li et al. (2010) showed that under higher light intensity, the neutral lipid synthesis pathway is redirected from starch synthesis. Rodolfi et al. (2009) also reported about 53% increase in fatty acid content of *Nannochloropsis* sp. with an increase of irradiance. Han et al. (2015) found a more than two-fold increase in the lipid content of *Chlorella* sp. from doubling the aeration rate of a bubble column photobioreactor; however, a gradual decrease was observed at three and four fold increases in air flow rate due to cell damage.

In this work, whilst biomass productivity was higher in the bubble column bioreactor, lipid volumetric production was at a maximum (0.19 $g_{Lipid}L^{-1}$) in the stationary phase of the gas-lift bioreactor sparged with CO_2-enriched air at the lower gas rise velocity. This compares to Vidyashankar et al. (2013) who achieved 0.15 $g_{Lipid}L^{-1}$ for *S. dimorphus* in a shear free photobioreactor with 5% CO_2 (v/v).

4.4 Conclusion

Experiments were conducted with the goal of comparing two gas-liquid contacting devices (gas-lift and bubble column) for growing microalgae in top-lit bioreactors. The aim was to be able to operate at depths greater than the typical 30 cm of commercial algal ponds, in particular if CO_2 enriched gas, such as industrial off-gas, is to be used to improve biomass productivity. Biomass productivity was found to be higher in the bubble

column, but the gas-lift bioreactor provided greater lipid volumetric productivity. The higher lipid content of the algal culture in the gas-lift bioreactor was likely induced by light stress as a result of the mixing pattern.

Top-lit gas-lift bioreactors have great potential for large-scale biodiesel production linked to (mitigating) industrial sources of CO_2. This is due to them being deeper and thereby providing both a smaller footprint on an industrial site and increased lipid productivity from enhanced CO_2 uptake. The hydrodynamics and energy requirements as studied in this study can be adapted to meet scalability requirements.

Chapter Five: Optimization of microalgae-sourced lipids for biodiesel production in a top-lit gas-lift bioreactor using response surface methodology

Paper #4 – Original Research, Energy (Submitted)

Nekoo Seyed Hosseini[1], Helen Shang[1], and John Ashley Scott[1*]

[1]Bharti School of Engineering, Laurentian University, Sudbury, ON, Canada

Abstract

Microalgae bioreactors that capture industrial carbon dioxide (CO_2) emissions to produce lipids for biodiesel are of significant interest. Sun-lit open raceways are generally considered the most economic method for mass cultivation, but the large physical footprint of these shallow systems can limit industrial site availability and gas transfer. To address these issues, a deep top-lit gas-lift bioreactor to culture microalgae and capture CO_2 was investigated. The results show a three times increase in areal biomass and lipid production when compared to traditional raceways used in large-scale microalgae production. Operational factors exerting significant effects on areal biomass productivity and areal lipid production were identified as gas flow rate, CO_2 content and dispersion height through the Plackett-Burman experimental design. By employing response surface methodology, models to predict areal biomass and lipid productivity were derived. The desirability function was then applied to obtain an optimal combination of operational parameters that maximize lipid production per unit area occupied by the bioreactor, while keeping the biomass production at a minimum in order to potentially reduce downstream processing costs. The optimum operational parameters that fulfills the requirements of the optimization function resulted in areal biomass productivity of 32.1 $g_{dw}m^{-2}d^{-1}$ and areal lipid production of 198.4 $g_{Lipid}m^{-2}$.

Keywords: Microalgae, Gas-lift, Top-lit, Footprint, Optimization, Biodiesel

5.1 Introduction

Use of industrial off-gas carbon dioxide (CO_2) to enhance the growth of microalgae, such as *Scenedesmus dimorphus,* which produce lipids suitable for transesterification into biodiesel (Y. Wang et al., 2016; Laamanen et al., 2014; Shang and Scott, 2011) not only provides a useful biofuel, but CO_2 emissions can also be mitigated. For this to occur on a large-scale, open ponds are still generally considered the most feasible option for microalgal cultivation (Amaro et al., 2012). These ponds take advantage of free sunlight energy, low mixing energy requirements, cost-effective construction and operation, and ease of scalability (Mendoza et al., 2013).

However, sun-lit open ponds need to be shallow at around 30 cm (Demirbas, 2010) due to light penetration limitations (Ono and Cuello, 2004), which restricts their areal biomass productivity. A low areal biomass productivity means a large physical footprint, which could significantly restrict their location on industrial sites near to off-gas sources. Furthermore, shallow depths will limit gas-liquid contact times when off-gas is bubbled in. As a solution to increase areal biomass productivity and reduce the physical footprint, one-meter deep top-lit gas-lift bioreactors have been proposed (Seyed Hosseini et al., 2015). These deeper bioreactors avoided the expense of sub-surface artificial lighting by using the gas-lift system and the greater depth improved gas-liquid transfer rates. The resulting areal biomass productivity for *S. dimorphus* of 60 $g_{dw}m^{-2}day^{-1}$ was significantly higher than the 5 to 45 $g_{dw}m^{-2}\,day^{-1}$ reported for traditional raceways and photobioreactors (Handler et al., 2012).

However, maximizing the level of suitable microalgal lipids, not overall biomass is key to biodiesel production. Irrespective of the type of bioreactor used, alteration of the biochemical composition of microalgal cells by manipulating growth simulators (Hoffmann et al., 2010), bioreactor design (Seyed Hosseini et al., 2016), and media formulation (Camacho-Rodríguez et al., 2015) are known as effective methods to favor the lipid production suitable for biodiesel. Growth conditions such as the availability of nutrients including nitrogen and sulfur (X. Zhou et al., 2014; Cakmak et al., 2012) the concentration of CO_2 (Xia et al., 2013), pH (Ertit Taştan et al., 2016; Eibl et al., 2014), light exposure including illumination area, photoperiod, light wavelength, and intensity (Yan et al., 2016; Liu et al., 2014), temperature (Xin et al., 2011) and hydrodynamic stress (Song et al., 2014) have been all reported as triggers of lipid synthesis in microalgal cells.

Some manipulation strategies, such as low pH, nitrogen deficiency, high irradiance, and high CO_2 concentration resulted in an increase in lipid content, but at the expense of total biomass concentration (Pruvost et al., 2011; Rodolfi et al., 2009; Widjaja et al., 2009). Therefore, in addition to an increase in lipid content, the relationship between lipid content and biomass concentration known as lipid production needs to be also considered.

The aim of this study was to systematically optimize the performance of a deep top-lit gas-lift bioreactor for a *Scenedesmus* sp. growth in terms of maximizing lipid production per unit area occupied by the bioreactor. This was done by evaluating the interactive effects of operational parameters, instead of using the traditional method of studying the effect of one individual parameter at a time. The aim is for the outcomes to help the

economics of biodiesel production, which simultaneously mitigates industrial CO_2 and minimizes the required site footprint. For the top-lit gas-lift bioreactor, therefore, various combinations of operational parameters including depth, aeration rate, CO_2 content, nutrient concentration, and initial biomass density resulted in different growth conditions that affected algal cell biomass concentration and lipid content.

There have been many studies on the effects of individual parameters such as pH, medium composition, and CO_2 concentration on the growth of *Scenedesmus* species. (Vidyashankar et al., 2013; Welter et al., 2013; Tang et al., 2011). The complexity of interactive effects among the factors on the lipid productivity has not, however, been widely studied. To address this, applying a statistical experimental design method, response surface methodology, especially when there is a possibility of interaction among the large number of components (Skorupskaite et al., 2015; Kirrolia et al., 2014; Dhingra et al., 2013), as an efficient strategy for the design of a microalgae cultivation system is proposed.

This current study was conducted in two steps. The first step, a factors screening phase, evaluated the influence of different parameters on algal growth and lipid accumulation through the application of a Plackett-Burman design. The second step, the optimization phase, was achieved by application of a response surface methodology. The optimum configuration of operational parameters to maximize lipid productivity was obtained, which was then verified with a top-lit gas-lift bioreactor.

5.2 Material and Methods

5.2.1 Microalgae strain and inoculum preparation

The freshwater microalgae *Scenedesmus dimorphus* were acquired from the University of Texas, Austin collection (1,237 UTEX collection). *Scenedesmus dimorphus* was selected due to its ability to grow under a wide range of CO_2 levels (Tang et al., 2011) and produce a higher lipid content compared to other microalgae, such as *Chlorella* sp. and *Chlorococcum* sp. (Vidyashankar et al., 2013). The stock culture was grown photoautotrophically and aseptically in Bold's Basal medium (BBM) (Andersen, 2005) and incubated at 25°C and 125 rpm. The stock culture was illuminated at 80 $\mu molm^{-2}s^{-1}$ on a photoperiod of 12 h light/12 h dark and supplied with Bold's Basal growth medium every three weeks.

5.2.2 The bioreactors configuration

The bioreactor used in this study was modified from previous study (Seyed Hosseini et al., 2015). It consisted of a concentric draft-tube gas-lift column with an internal diameter (D_i) of 13 cm and height of 80 cm, secured in an outer column with a diameter (D_o) of 20 cm. They were made from 5 mm thick, transparent plexiglas. The draft tube was located 5 cm from the bottom and gas was sparged in the draft tube as a riser section (Figure 5.1). The ratio of cross sectional area of riser to downcomer was 0.83 for the gas-lift reactor. The bioreactor had side ports at 5 cm and 50 cm from the base for taking samples. The depth of the bioreactor was varied by changing the dispersion height (Δh), which is the distance from the riser to the surface of the culture medium. Air mixed with carbon dioxide was sparged through a 10 cm diameter ceramic sparger with a mean pore size of

15 μm (Refractron Technologies Corp., NY, USA). The flow rate was controlled by using rotameters (±5%) (Omega Engineering Ltd., QC, Canada).

The anticipated application of the gas-lift system is for it to be installed, as with traditional commercial raceways, by burying it so that only the top surface is exposed. Therefore, the outside of the bioreactor was covered with a layer of black plastic sheet on top of a white plastic sheet to block light entrance from the side. Light energy for the photosynthesis was provided only from the top of the bioreactor to simulate buried open systems on an industrial site, which is top lit by sunlight only. A 90 watt circular grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) was used providing a 12 h light/ 12 h dark photoperiod. The photosynthetic active radiation at the surface of the culture was kept around 1,000 $\mu molm^{-2}s^{-1}$ by changing the distance between the light source and culture medium surface. The surface of the culture medium received the highest light intensity, which decreased exponentially with depth. Measurements of the light intensity transition from the light to dark region were carried out using a light meter (±0.4%) (LI-250 A, LI-COR Biosciences, NE, USA) equipped with a quantum sensor (±5%) (LI-193SA, LI-COR Biosciences, NE, USA) at increments of known distances below the surface. An intensity of 50 $\mu molm^{-2}s^{-1}$ was considered the minimum for the light zone (Barbosa et al., 2003b) to estimate light fraction (ξ) (Eq. 1) as a ratio of time algal cells spend in the light section (t_l) over the mean circulation time (t_c). The mean circulation time was calculated by measuring the time taken for a 5 mm colored tracer bead (Engineering Laboratories, NJ, USA) with the same density as water to circulate one cycle through an arbitrarily chosen horizontal reference plane in the bioreactor.

$$\xi = \frac{t_l}{t_c} \quad (1)$$

Make up water was supplied every day to compensate for evaporative losses in order to keep the dispersion height at specified level and experiments were carried out at 22±2°C.

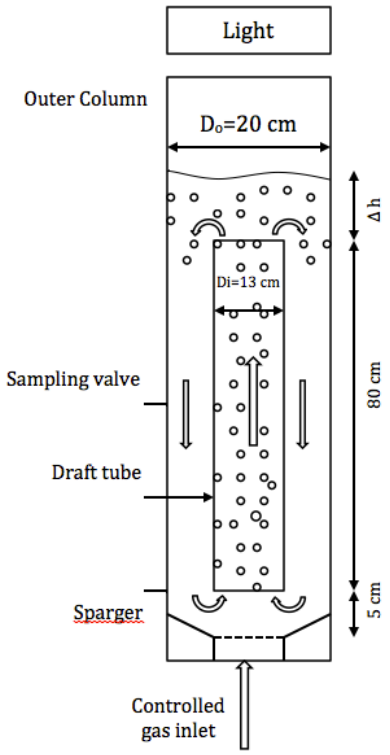


Figure 5.1: Schematic diagram of the top-lit gas–lift bioreactor

5.2.3 Experimental design

This study was conducted in two phases. The first phase, factors screening, employed a two-level, five-factor Plackett-Burman (PB) design (Plackett and Burman, 1946) aimed to evaluate the significance of operational parameters on biomass and lipid production. The five independent factors tested in the first phase were: initial biomass density (X_1), gas flow rate (X_2), CO_2 content of feed gas (X_3), dispersion height (X_4) and media

composition (X_5). An operational range was selected for each factor and the minimum and maximum levels of each factor over the studied operational range were coded as -1 and +1, respectively. The initial biomass density was determined spectrophotometrically so as to provide a starting absorbance of the culture at 550 nm (A_{550}) of 0.1 cm^{-1} and 0.3 cm^{-1} as the low and high levels. This absorbance range was equivalent to biomass densities of 0.02 to 0.08 $g_{dw}L^{-1}$. The gas flow rate was in the range of 4-14 $Lmin^{-1}$ in order to provide a light fraction allowance of 50%-80% (Seyed Hosseini et al., 2015). The CO_2 content of feed gas was varied between 0.038% (CO_2 content of air) and 6%, the latter to simulate the CO_2 concentration of a nickel smelter's off-gas (Shang and Scott, 2011). The dispersion height above the riser was varied within the range of 10 to 30 cm. For the media composition, "+1" represents the concentration of media components of standard Bold's Basal medium (BBM), and "-1" represents a concentration of half of those in standard Bold's Basal medium.

This first experimental phase consisted of four blocks (runs) of nine bioreactors. Blocking was applied due to the limitation of experimental set-up to run at the same time. The experimental design presented in Table 5.1 was conducted in triplicate. The mean values of response functions of interest, areal biomass productivity (P_a) and areal lipid production (P_{La}) were analyzed statistically using Minitab-16 software. The significant factors at a confidence level of 95% (p-value < 0.05) by using regression analysis were identified as gas flow rate (X_2), CO_2 content of feed gas (X_3), and dispersion height (X_4), and further optimized in the second phase by applying response surface method (RSM). Response surface method was employed to determine an optimal combination of significant factors by constructing a five-level, three-factor, full factorial central

composite design (CCD). This design used the three most important factors obtained from the screening phase. The range over which the factors were changed was increased to elicit more significant responses. The optimization experimental phase according to the central composite design (CCD) consisted of 20 runs (Ryan, 2007), which were conducted in three blocks due to the number of available bioreactors to run at the same time. According to the CCD method, three groups of design points including 8 factorial points (also called cubic points), 6 axial points (also called star points), and 2 center points for each block (Ryan, 2007) were used for finding the coefficients of the predictive regression model. The obtained responses from the CCD design were then fitted to the following predictive quadratic polynomial model (Eq. 2) to provide an approximation for response surfaces over the studied ranges of factor variations:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad (2)$$

where Y is the predicted response, β_0 the independent coefficient, β_i the linear coefficient associated to each independent factor X_i, and β_{ii} and β_{ij} $(i \neq j)$ the coefficients for quadratic and interaction effects of X_i and X_j, respectively. These constant regression coefficients were determined by the method of least squares.

A summary of the methodology used in this study is presented in Figure 5.2

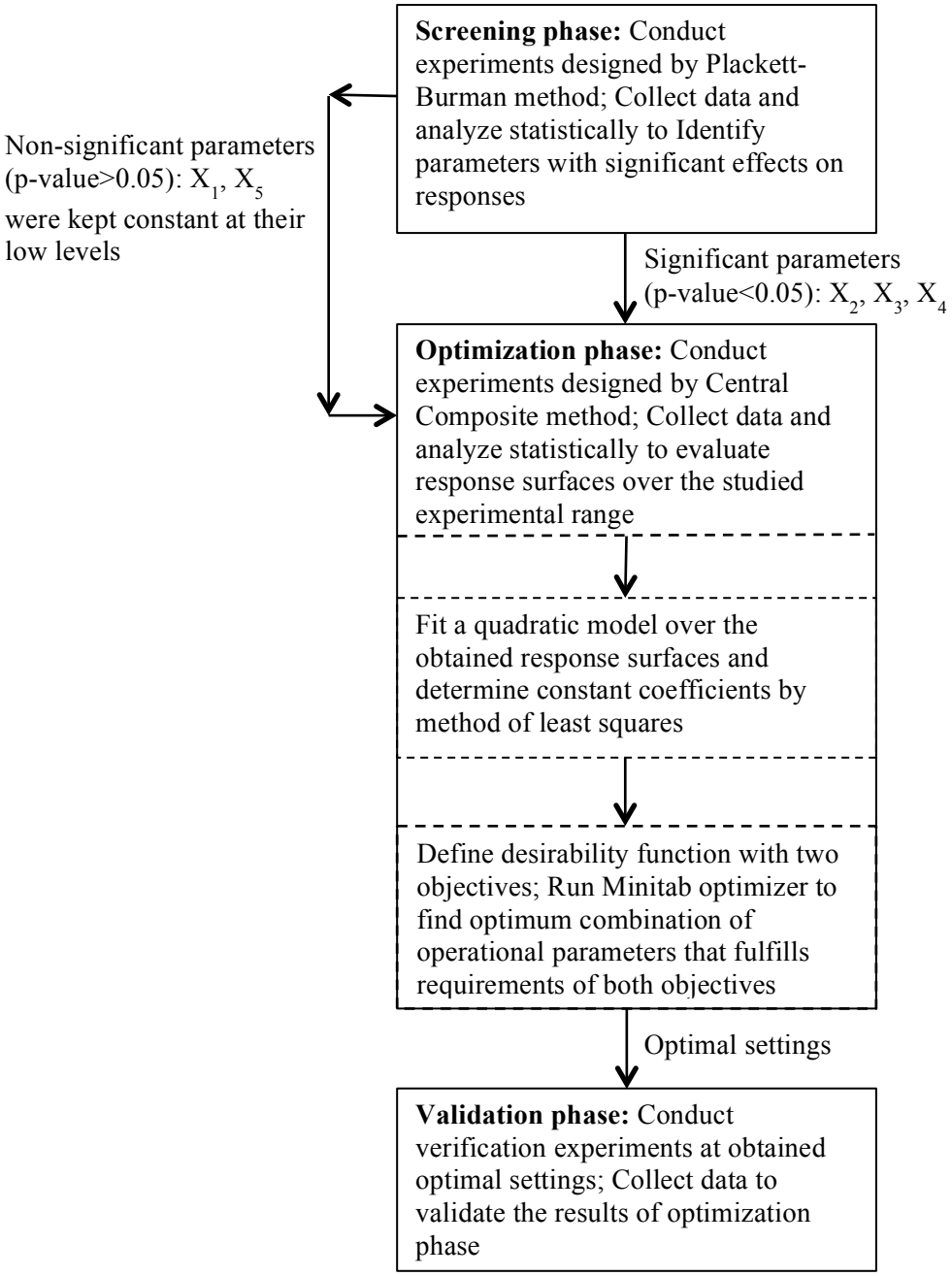


Figure 5.2: A summary of the methodology

5.2.4 Analytical procedures

Microalgal growth was tracked by measuring absorbance using a spectrophotometer (UV-1700 UV-VIS, Shimadzu, Tokyo, Japan) at 550 nm wavelength. Dry biomass concentration (C_b) was determined by vacuum filtration of 100 ml of algal culture through glass microfiber filter paper (Grade 151, Ahlstrom Filtration LLC, PA, USA) followed by oven-drying over night at 60°C.

Areal biomass productivity, P_a, was calculated as follows (Eq. 3) (Andersen, 2005) from the initial (C_{bi}) and final (C_{bf}) dry biomass concentrations over the duration of exponential growth phase (Δt):

$$P_a = \frac{1000\,(C_{bf} - C_{bi})\,d_t}{\Delta t} \qquad (3)$$

where d_t is total bioreactor depth.

With the aim of determining lipid production per unit area occupied by the bioreactor, the lipid content of the microalgae was extracted first through a modification of the method described by Folch et al. (1957). Algal biomass was harvested by centrifugation, frozen at -80°C and subsequently freeze-dried at -50°C under vacuum for 24 h. Freeze-dried biomass was then mixed with 1.2 mL of chloroform:methanol (2:1 v/v) in a centrifuge tube and sonicated using a Sonic Dismembrator Model 500 (Fisher Scientific, Ottawa, Canada) for 30 minutes. The samples were centrifuged using an Allegra X-15R Centrifuge (Beckman, Palo Alto, CA) and the supernatant was removed into a weighed tube. Extraction was repeated in triplicate with the collected solvent layers being combined. The solvent was then evaporated in a vacuum drier and the mass of the

remaining lipid determined gravimetrically. The mass of the lipid fraction was used to measure lipid content (θ_L) of algal cells (% $g_{Lipid}/g_{dw,Biomass}$).

Areal lipid production (P_{La}) (Eq. 4) was calculated by the product of biomass concentration (C_b , g_{dw}/L) and lipid content (θ_L, % $g_{Lipid}/g_{dw,Biomass}$) as follows:

$$P_{La} = \frac{1000 C_b \theta_L V_t}{A_t} \quad (4)$$

where C_b is the biomass concentration at the end of the stationary phase, θ_L is the percentage of microalgae cell biomass that is lipid, V_t is the total volume of the bioreactor and A_t the area occupied by the bioreactor.

5.3 Results and discussion

5.3.1 Factor screening phase

In order to examine the effect of the operational parameters of initial biomass density (X_1), gas flow rate (X_2), CO_2 content of feed gas (X_3), dispersion height (X_4) and media composition (X_5) on *Scenedesmus* sp. biomass synthesis and lipid accumulation, a PB design consisting of 12 experiments was built and run in triplicate. Statistical analysis was implemented on the average values of obtained responses for areal biomass productivity (P_a) and areal lipid production (P_{La}). Table 5.1 presents the experimental configurations suggested by the PB design for each treatment and the obtained average values of triplicate experiments for P_a and P_{La}. By employing quadratic least squares, the terms of the model were obtained, and the root mean square error of the quadratic least squares analysis then calculated for each response.

Table 5.1: Experimental design and results using the Plackett-Burman method to evaluate the significance of operational factors

Run	Factors					Responses	
	X_1	X_2	X_3	X_4	X_5	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	Areal lipid production ($g_{Lipid}m^{-2}$)
1	+1	-1	+1	-1	-1	35.3	158.7
2	+1	+1	-1	+1	-1	20.8	86.3
3	-1	+1	+1	-1	+1	38.4	184.3
4	+1	-1	+1	+1	-1	17.4	89.8
5	+1	+1	-1	+1	+1	22.2	75.8
6	+1	+1	+1	-1	+1	33.5	175.8
7	-1	+1	+1	+1	-1	39.3	186.3
8	-1	-1	+1	+1	+1	19.6	95.5
9	-1	-1	-1	+1	+1	6.7	51.3
10	+1	-1	-1	-1	+1	24.1	67.5
11	-1	+1	-1	-1	-1	23.6	95.9
12	-1	-1	-1	-1	-1	23.3	77.9

With regards to areal biomass productivity, by applying analysis of variance, three factors including X_2 ($p = 0.049$), X_3 ($p = 0.024$) and X_4 ($p = 0.047$) were found significant at a confidence level of 95%. Similar factors were observed to have significant effect on the areal lipid production with a *p*-value of 0.008, 0.001 and 0.004 for X_2, X_3, and X_4, respectively. This can be explained by the effect of these factors on CO_2 transfer rate to algal cells and consequently impacting the rate of biomass doubling time and lipid synthesis. The R^2 corresponding to the areal biomass productivity and lipid production models were 78% and 91%, representing a good fit for the responses.

Initial biomass density (X_1) with a *p*-values of 0.912 for areal biomass productivity and 0.717 for areal lipid production, over the studied range did not have significant effect on any of the responses. Giordano et al. (2014) reported similar observations with half an order difference in the inoculum cellular density for growing *Chlorella* sp.

Media composition, (X_5), with a *p*-values of 0.494 for areal biomass productivity and 0.760 for areal lipid production, did also not show an influence on any of the responses. The study of Welter et al. (2013), which provided the minimal medium for optimal growth and lipid production of *Scenedesmus dimorphus*, also showed insignificant effects from reduction of chemical concentration of 3N-Bold Basal medium to 1/6, with the exception of potassium phosphate. The cost of media preparation contributes significantly in commercial scale cultivation of algae for biodiesel production, and therefore, being able to use half of the concentration of nutrients would be more economic and environmentally worthwhile.

The factors to be taken into consideration in the optimization phase are gas flow rates, CO_2 content, and dispersion height. Factors that were demonstrated to be non significant were kept fixed at their low levels. This meant that initial biomass density was at an absorbance (A_{550}) of 0.1 cm^{-1} and media composition was half the concentration of standard BBM.

5.3.2 Optimization phase

After identifying the significant factors for cultivation of *S. dimorphus* in the top-lit gas-lift bioreactor in the screening phase, a five-level, three-factor central composite design consisting of 20 experiments was constructed. The factors included in this design were gas flow rate (X_2), feed gas CO_2 content (X_3) and dispersion height (X_4). The coded levels defined by standard central composite design (Ryan, 2007) and the actual levels of the three significant factors are presented in Table 5.2. The axial points (-1.633 and +1.633) are calculated based on the requirement of blocking in the central composite

design. This requirement is that the sum of squares of the individual factor coordinates must be proportional to the block size (Ryan, 2007). All cultures were developed with a half concentration of standard BBM and the initial cellular density related to the absorbance (A_{550}) of 0.1 cm^{-1}, over a period of three weeks.

Table 5.2: Coded and actual levels of significant factors designed through CCD approach

Code	Factor	Coded levels				
		-1.633	-1	0	+1	+1.633
X_2	Gas flow rates (L/min)	5.5	8	12	16	18.5
X_3	CO_2 content (%v/v)	0.1	2	5	8	9.9
X_4	Dispersion height (cm)	14	20	30	40	46

The factor combinations suggested by the CCD experimental plan and the obtained responses for areal biomass productivity (P_a) and areal lipid production (P_{La}) are presented in Table 5.3.

Table 5.3: Experimental design and results using the central composite design to find the optimal operational variables for algal cultivation and lipid production

Run	Factors Assignment			Responses	
	X_2	X_3	X_4	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	Areal lipid production ($g_{Lipid}m^{-2}$)
1	-1.633	0	0	16.6	102.4
2	0	-1.633	0	20.7	79.8
3	+1.633	0	0	26.5	195.5
4	0	0	0	44.4	241.5
5	0	+1.633	0	41.6	195.5
6	0	0	0	48.1	230.3
7	0	0	-1.633	32.3	138.2
8	0	0	+1.633	21.9	102.1
9	0	0	0	43.1	184.7
10	+1	-1	+1	19.5	147.6
11	+1	+1	-1	28.4	194.3
12	-1	-1	-1	25.2	90.4
13	-1	+1	+1	22.8	68.8
14	0	0	0	45.7	230.7
15	-1	+1	-1	38.8	199.5
16	-1	-1	+1	14.6	62.5
17	+1	+1	+1	29.3	200.9
18	+1	-1	-1	23.2	115.5
19	0	0	0	50.5	253.8
20	0	0	0	46.5	207.6

All responses were adjusted with quadratic models and the constant regression coefficients were determined by the method of least squares. Table 5.4 summarizes the results obtained by applying analysis of variance (ANOVA) to the experimental data. Through analyzing the mathematical models, it can be confirmed that the three examined factors and their quadratic effects were significant (p-value < 0.05) for both responses.

As can be seen in Table 5.4, with regards to the areal biomass productivity, only the interaction of the gas flow rate and dispersion height (X_2X_4) was significant ($p = 0.028$) and exerted a positive effect on the response. This can be explained by the fact that both

gas flow rate and dispersion height have direct influence on the residence time of gas bubbles in the culture, which consequently affects the CO_2 transfer rate into the medium.

As can be seen in Table 5.4, with regards to areal lipid production, two interactions had a significant effect: X_2X_4 with a *p*-value of 0.010 exerting a positive effect and X_3X_4 with a *p*-value of 0.053 exerting a negative effect. The significant interaction of gas flow rate and dispersion height (X_2X_4) is due to the compound influence of these factors on liquid circulation time and frequency.

The culture circulation frequency is related to the continual circulation of algal cells between the light and dark zones, and thereby the cells being exposed to the "flashing light" effect that has been shown to induce the lipid synthesis (Abu-Ghosh et al., 2016). For example, Choi et al. (2015) reported an increase in total fatty acids content of an *Obliquus* species due to a 5 times per minute flashing light stress. The absorption rate of CO_2 into the culture medium depends on the medium volume and the gas residence time. This is demonstrated by the significant interaction effect of CO_2 content of the sparged gas and dispersion height (X_3X_4). In Table 5.4, the significant factors were only included in the regression models in order to improve the predicted models (Ryan, 2007).

Table 5.4: Analysis of variance for response surface quadratic models

Factor	P_a ($g_{dw}m^{-2}day^{-1}$)		P_{La} ($g_{Lipid}m^{-2}$)	
	Regression coefficient	*p*-value	Regression coefficient	*p*-value
Regression coefficients including all factors and their second-order interactions				
Constant	**-93.52**	**0.001**	**-381.7**	**0.010**
Blocks	0.249	0.781	**3.239**	0.588
X_2	**11.99**	**0.000**	**26.94**	**0.038**
X_3	**8.789**	**0.001**	**58.47**	**0.001**
X_4	**2.963**	**0.002**	**16.02**	**0.006**
X_2X_2	**-0.566**	**0.000**	**-1.626**	**0.002**
X_3X_3	**-0.607**	**0.000**	**-3.361**	**0.001**
X_4X_4	**-0.070**	**0.000**	**-0.368**	**0.000**
X_2X_3	-0.071	0.474	0.174	0.789
X_2X_4	**0.074**	**0.028**	**0.617**	**0.010**
X_3X_4	-0.003	0.932	**-0.535**	**0.053**
Regression coefficients including significant factors for reduced model				
Constant	**-88.25**	**0.000**	**-385.3**	**0.003**
X_2	**11.64**	**0.000**	**27.81**	**0.016**
X_3	**7.839**	**0.000**	**60.56**	**0.000**
X_4	**2.946**	**0.000**	**16.02**	**0.002**
X_2X_2	**-0.566**	**0.000**	**-1.626**	**0.001**
X_3X_3	**-0.607**	**0.000**	**-3.361**	**0.000**
X_4X_4	**-0.070**	**0.000**	**-0.368**	**0.000**
X_2X_4	**0.074**	**0.013**	**0.617**	**0.005**
X_3X_4	N/A	N/A	**-0.535**	**0.043**

- Bold values represent statistically significant results

The probability values of both models were smaller than 1 x 10^{-4} and neither of the two models had significant lack of fit, showing a high prediction reliability. Blocking also did not show significant effect on the responses. Equations (5) and (6) are reduced mathematical presentations of the responses which only include the significant factors:

$$P_a = -88.25 + 11.64X_2 + 7.84X_3 + 2.95X_4 - 0.57X_2^2 - 0.61X_3^2 - 0.07X_4^2 + 0.07X_2X_4 \quad (5)$$

$$P_{La} = -385.32 + 27.81X_2 + 60.56X_3 + 16.02X_4 - 1.63X_2^2 - 3.36X_3^2 - 0.37X_4^2 + 0.62X_2X_4 - 0.54X_3X_4 \quad (6)$$

The coefficients of determination (R^2) corresponding to the predictive models were 87.1% and 85.2% for P_a and P_{La}, confirming that variability of the system could be accurately predicted by the model.

Both biomass and lipid production are favored by increasing the volumetric gas flow rate, CO_2 content of the feed gas, and dispersion height. In a previous study (Seyed Hosseini et al., 2016), gas flow rate exerted a negative effect on the areal productivity and lipid accumulation at a fixed depth of 100 cm. In this study, however, the depth of the bioreactor is varied and as mentioned earlier, the compound effect of gas flow rate and depth showed significant positive impact on both responses. This result is in agreement with the study of Khoo et al. (2016), which concluded that increasing the sparging rate up to a critical level had a positive effect on biomass productivity, but increasing the rate above the critical level had a negative effect on productivity.

The positive effect of CO_2 concentration on lipid synthesis by it causing a lower media pH, has been also previously demonstrated (Seyed Hosseini et al., 2016). Improved biomass formation due to increasing the CO_2 concentration is also in agreement with study of Kumar and Das (2012) who reported enhanced biomass productivity when increasing the CO_2 concentration up to 10% in sparged gas. Increasing the dispersion height, which means a higher medium depth, results in longer gas-liquid contact time and consequently increased mass transfer rate of CO_2. This confirms that applying the gas-lift

system in a deep cultivation system could help overcome light penetration restrictions in open systems used for cultivating microalgae.

The main effects of the tested factors and the mutual effects of factors over responses are demonstrated in Figure 5.3. Although all three factors have positive effects on the responses, the maximum value of areal biomass and lipid productivity were not obtained at the highest level of the factors due to the significant interaction effects between them. As can be seen in Figure 5.3A and C, an increase in gas flow rate up to a critical level resulted in an increase in biomass and lipid production. This critical level depends on the gas rising rate and dispersion height (consequently the overall bioreactor depth). This observation is similar to the study of Barbosa et al. (2004) which stated that cell death rate increased with increasing gas entrance velocity, but it decreased with increasing the culture height.

A comparable behavior was observed in Figure 5.3B and D, which present the interaction of feed gas CO_2 content and bioreactor dispersion height. While the gas flow rate was kept constant, biomass and lipid production improved when the CO_2 concentration was increased up to a critical level, which is dependent on the culture medium volume. Increasing the CO_2 concentration higher than that critical level resulted in a decrease in algal growth and lipid synthesis. This can be explained by a decreased pH level of the culture medium from higher levels of carbonic acid inhibiting RuBisCO (ribulose 1,5-bisphosphate carboxylase / oxygenase) activity in the CO_2 concentrating mechanism (CCM) (Zhao and Su, 2014).

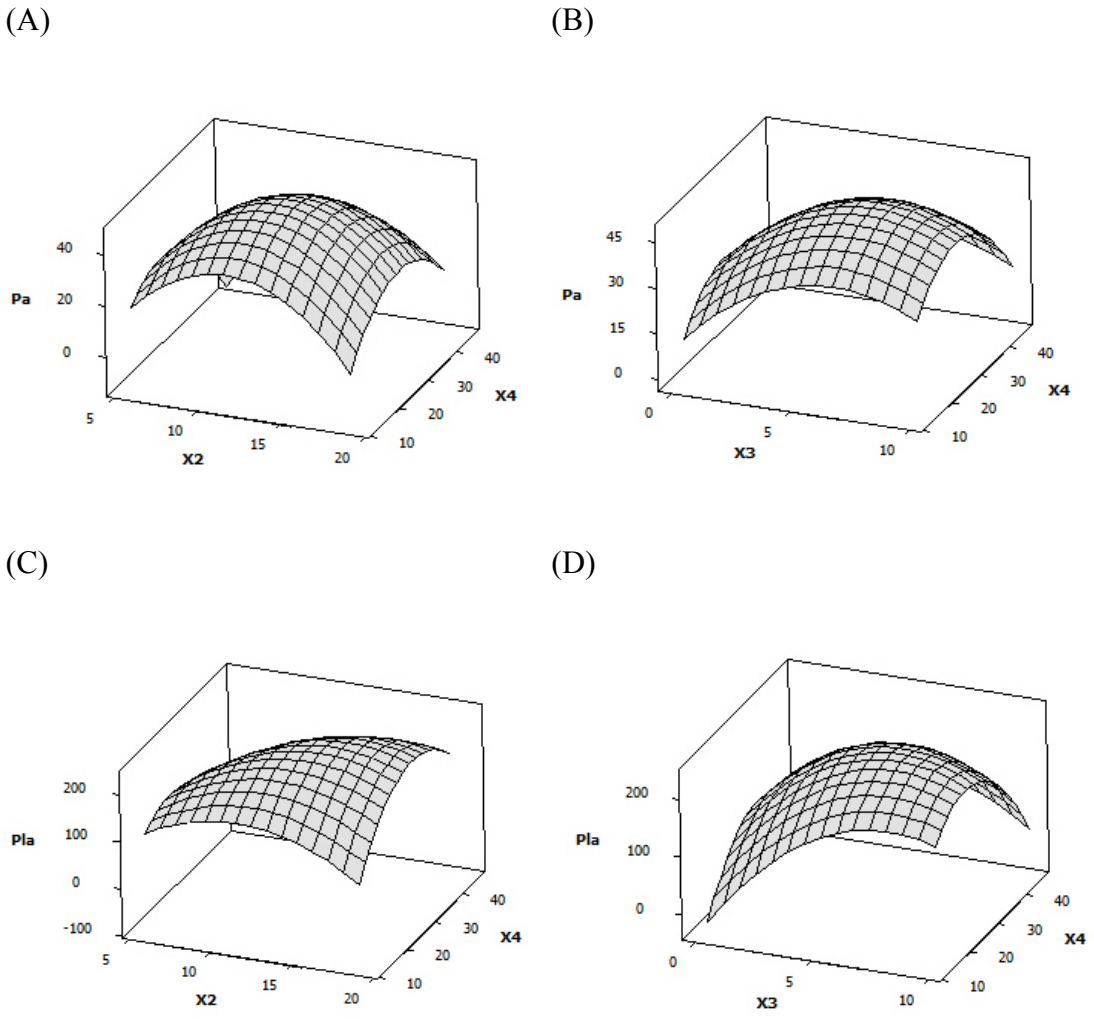


Figure 5.3: Response surfaces corresponding to areal biomass productivity (P_a - $g_{dw}m^{-2}$ day^{-1}) and lipid productivity (P_{La} - $g_{Lipid}m^{-2}$) as a function of the gas flow rate (X_2 – L/min) and dispersion height (X_4 - m) (A and C, respectively), and as a function of CO_2 content of feed gas (X_3 - %v/v) and dispersion height (X_4 - m) (B and D, respectively). The third factor was kept constant at its medium level for all figures.

The next step was finding the optimal configuration of the operational parameters by using a desirability function (D) that meets the fulfillment of the set requirements. As the key role in biodiesel production is the production of lipids, the main objective of the optimization was set to maximize the P_{La}. At the same time, another goal would be to keep biomass production low as possible while still producing the highest amount of

lipids. This would help in reducing downstream processing costs such as microalgae harvesting, drying and lipid extraction. The desirability function for theses two objectives was created using Minitab-16 software, therefore, to provide the best compromise solution for satisfying the goals relative to each output (Ryan, 2007).

Table 5.5 summarizes the criteria applied to the desirability function and the optimal combination suggested to achieve maximum lipid production.

Table 5.5: Criteria used in the optimization of multiple responses and suggested optimal combination of factors

Factors and Responses	Optimization Criterion	Lower Limit	Upper Limit	Optimal Level
X_2 (L/min)	In range	5.5	18.5	17.7
X_3 (%v/v)	In range	0.1	9.9	6.4
X_4 (cm)	In range	14	46	32.5
P_a ($g_{dw}m^{-2}day^{-1}$)	Minimize	14.6	50.5	N/A
P_{La} ($g_{Lipid}m^{-2}$)	Maximize	62.5	253.8	N/A

With the suggested optimal combination in Table 5.5, the corresponding predicted values of responses were 222.5 $g_{Lipid}m^{-2}$ and 30.4 $g_{dw}m^{-2}day^{-1}$, with desirability index of 0.97.

It can be concluded that a 1.17 m deep bioreactor with an initial concentration at an absorbance (A_{550}) of 0.1 cm^{-1}, fed with half concentration of standard BBM, sparged with 6.4% CO_2-enriched air at a gas flow rate of 17.7 $Lmin^{-1}$ would achieve the highest lipid production per unit area occupied by the bioreactor along with lower biomass production and accordingly lower downstream costs.

5.3.3 Validation phase

The optimal combination of factors was verified experimentally. In order to evaluate the results, *Scenedesmus* sp. was cultured in three bioreactors with operational parameters corresponding to the optimal combination found in the previous section. Figure 5.4 presents the growth profile and areal lipid production variations of cultures over three weeks of experiments. The error bars indicate the standard error of triplicate observations, which can be explained due to uncertainties of measuring instruments, sensitivity of models to operational parameters or random error in biological systems. The experimental values for areal biomass productivity and lipid production per unit area occupied by the bioreactor were 32.1 $g_{dw}m^{-2}day^{-1}$ and 198.4 $g_{Lipid}m^{-2}$, respectively. These experimental results agreed within ±10% of the predicted responses and confirmed the predicted optimal combination of factors successfully allowed maximizing lipid production. The suitability of the obtained models (Eq. 5 & 6) to present the studied process was also confirmed.

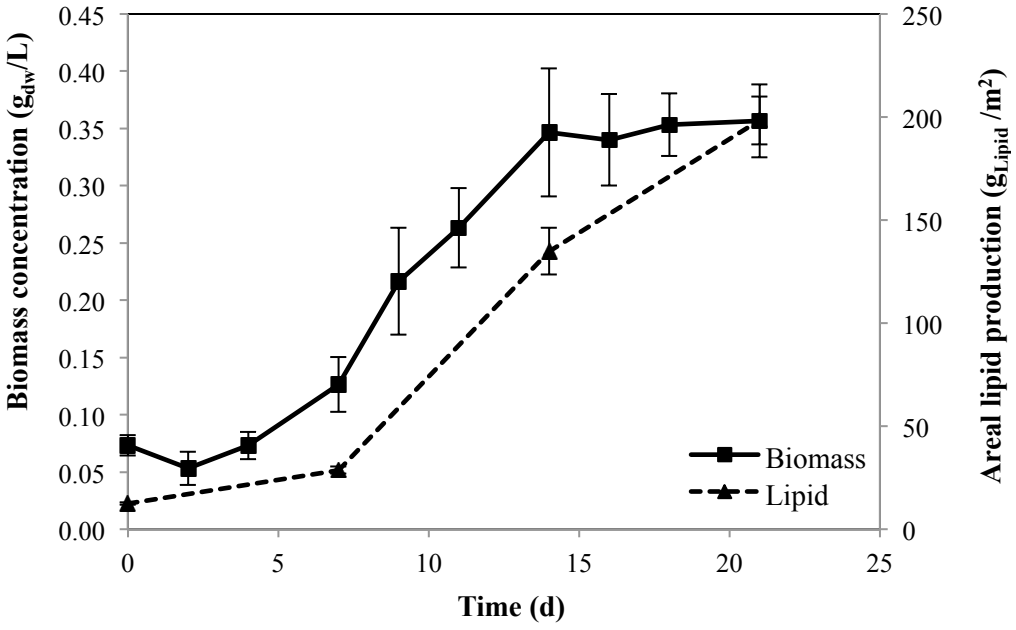


Figure 5.4: Growth profile and areal lipid production of *Scenedesmus* sp. in 1.17 m deep top-lit gas-lift bioreactors sparged with 6.4% CO_2-enriched air at gas flow rate of 17.7 L/min. Error bars indicate the standard error of triplicate observations.

The pH of the bioreactors over the three-week experimental run dropped from 6.7 to 5.7 and no fresh media was added over the run of the experiment. The light fraction was measured as 58%, which was in agreement with the results of Janssen et al. (2000) who stated that the optimal conditions for algae require 20% of the systems cycle to be in the dark region and that efficiency decreases significantly when the time within the dark zone is above 50% of the overall cycle time. The highest areal biomass productivity achieved at the end of the stationary phase was 56% higher than typical areal biomass productivities of shallow raceways (20 $g_{dw}m^{-2}day^{-1}$) (Seyed Hosseini et al., 2015).

The areal lipid production increased significantly when microalgae cells entered the stationary phase. This was likely due to flashing light stress, nitrogen deficiency and shear stress in the top-lit gas-lift bioreactors. This is in agreement with studies of Choi et

al. (2015), Song et al. (2014) and Cakmak et al. (2012). Although the biomass density did not change significantly during the stationary phase, the lipid content of algal cells did increase over one week leading to a 47% increase in areal lipid production. However, considering the yearly production of lipids from the algal cultivation facility, harvesting the biomass and extraction of lipid at the end of the exponential phase resulted in 4.1% more lipid production per square meter compared to harvesting at the end of the stationary phase at the expense of 9 more batch runs. Therefore, depending on the cost of downstream processes, an economical analysis would be required to decide about the best harvesting time for the algal system.

5.3.4 Sensitivity analysis

The impacts of the variation of individual operational parameters from the optimum setting predicted by the model, are presented in Figure 5.5. For all the plots, the other two parameters are kept constant at their optimum levels. Variation of each parameter was limited to the studied experimental range. As can be seen, the models are more sensitive to gas flow rate (X_2) and dispersion height (X_4) compared to CO_2 concentration over the studied experimental range. This can be explained due to the complex interaction among parameters, which has significant effects on areal biomass and lipid productivities as showed in section 5.3.2.

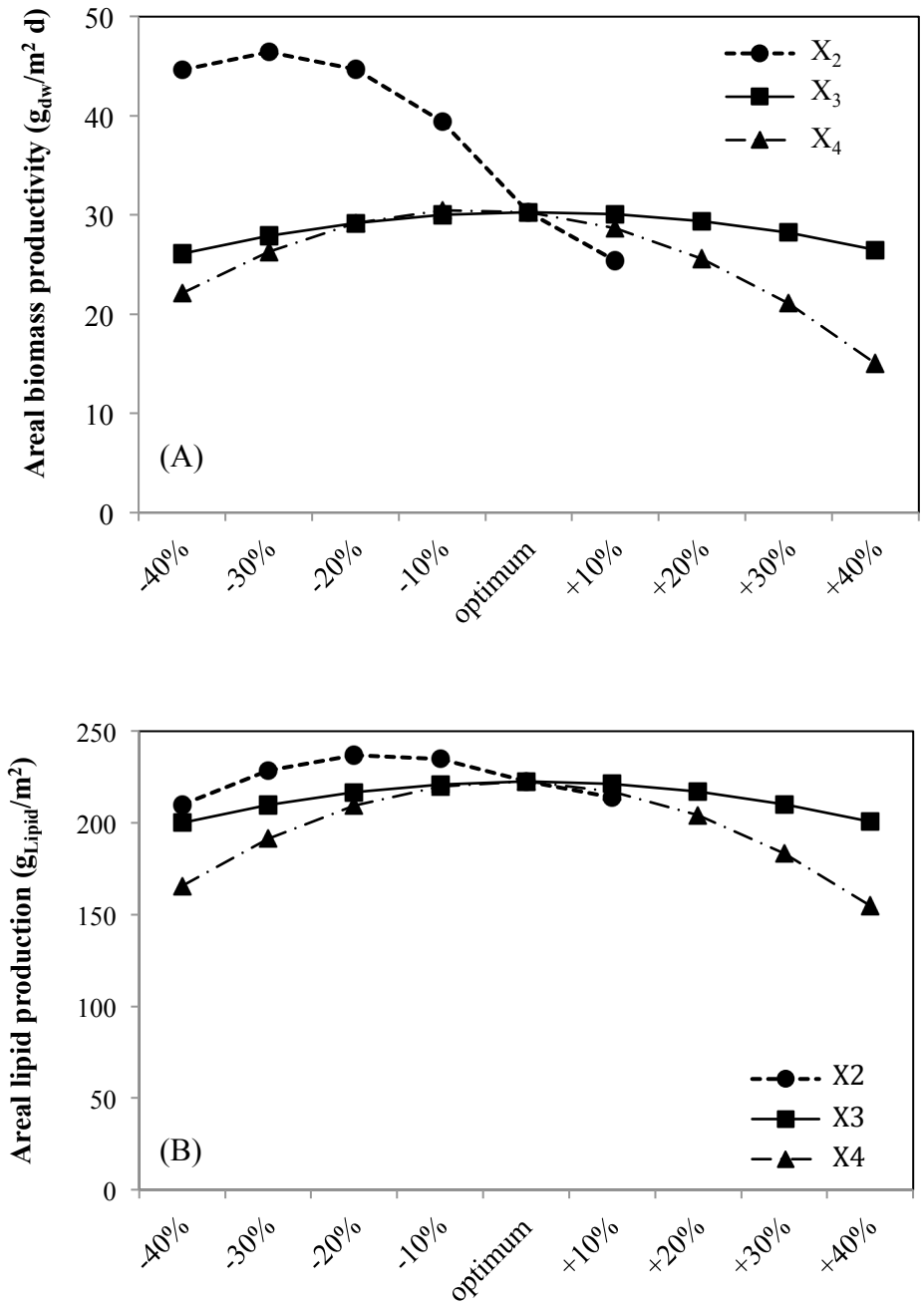


Figure 5.5: Models sensitivity analysis corresponding to (A) areal biomass productivity (P_a - g_{dw}/m^2d) and (B) areal lipid production (P_{La} - g_{Lipid}/m^2) as a function of the gas flow rate (X_2 – L/min), dispersion height (X_4 - m) and CO_2 content of feed gas (X_3 - %v/v)

5.4 Conclusion

Developing a top-lit gas-lift bioreactor with optimal operational parameters could help the mass production of microalgal lipids suitable for transesterification into biodiesel. In particular it offers a means by which to reduce the physical footprint of biomass production facilities housed on industrial sites for capture and mitigation of CO_2 emissions. From this work a 1.17 meter deep top-lit gas-lift bioreactor sparged with 6.4% CO_2-enriched air provided 32% higher lipid production per unit area occupied by the bioreactor (198.4 $g_{Lipid}\, m^{-2}$) than traditional shallow ponds.

A systematic optimization approach to evaluate the interactive effects of operational parameters on microalgae cells biomass and lipids productions was applied. Mathematical models for biomass and lipid production were developed and analyzed statistically. A two-objective optimization function was built to evaluate the optimum combination of operational parameters that fulfills the requirements of both lipids and biomass productions. From these it was determined that growth media with a half concentration of standard nutrients, along with an optimum combination of operational factors, provided the highest lipid productivity per unit area occupied by the bioreactor. It also helped reduce the amount of biomass produced while keeping the highest lipid production, thereby potentially improving the economics of downstream processing.

Chapter Six: Increasing productivity of a top-lit deep open microalgal cultivation system for biodiesel production by using a light column

Paper #5 – Original Research, Biomass & Bioenergy (Submitted)

Nekoo Seyed Hosseini[1], Helen Shang[1], and John Ashley Scott[1*]

[1] Bharti School of Engineering, Laurentian University, Sudbury, ON, Canada

Abstract

Mass cultivation of microalgae aided by mitigation of carbon dioxide (CO_2) emissions from industrial off-gases to produce lipids as a biodiesel feedstock can help environmental sustainability. Traditional open microalgae cultivation systems that use sunlight as a source of energy to derive photosynthetic reactions have a low light utilization efficiency due to their shallow depth, resulting in limited areal biomass and lipid productivities. To address these issues, a non-energy-consuming light column was employed in a top-lit gas-lift bioreactor. The biomass and lipid productivity per unit area occupied by the bioreactor was increased by 33% and 16%, respectively when using the light column. The factors influencing the light and CO_2 absorption rates were investigated with a Plackett-Burman experimental design in the bioreactor coupled with the light column. The mathematical models for predicting the biomass and lipid production of the bioreactor as well as optimal configurations for maximizing lipid production and minimizing production costs were determined by applying the response surface methodology.

Keywords: Microalgae, Gas-lift, Top-lit, Light column, Areal productivity, Biodiesel

6.1 Introduction

Microalgae are autotrophic microorganisms that have shown great potential as a feedstock for biodiesel, bioethanol and other bioactive value-added compounds. Furthermore, cultivation of microalgae for nutrient removal from wastewater and in particular carbon dioxide (CO_2) sequestration of industrial off-gases has attracted significant interest.

Photosynthetic algal species are light-driven cells that can convert light energy to chemical energy by absorbing nutrients, carbon sources and light energy (Solimeno et al., 2015). It has been proposed that the CO_2 in the emissions of industrial off-gas as a free source of carbon can be captured by microalgal cells for photosynthesis and simultaneously be mitigated to reduce green house gas emissions (Lara-Gil et al., 2016; Laamanen et al., 2014). Studies have been conducted to evaluate the absorption rate of CO_2 from actual or simulated off-gases and its influence on algal growth rate and biochemical compositions of biomass (Posada et al., 2016; Seyed Hosseini et al., 2015).

For a light source, sunlight energy and artificial lighting have both been utilized to stimulate photosynthetic reactions (Brindley et al., 2016). The light source (Orefice et al., 2016), intensity (Přibyl et al., 2016), spectra (Vadiveloo et al., 2015), light-dark photoperiods (Y. E. Cheah et al., 2015), frequency (Maroneze et al., 2016), and light-exposed surface area (X. Zhou et al., 2014) have all been widely reported to have significant impact, in particular on microalgae biomass formation rate and the biochemical content, including lipids, carbohydrates, proteins, vitamins, pigments and antioxidants (Pulz and Gross, 2004).

The effectiveness of any microalgal-sourced process depends on maintaining low production costs whilst achieving the highest level of desired biochemical content (Maroneze et al., 2016). Production costs include the operation and maintenance of cultivation systems, labor, raw materials and energy expenses. Manipulation of the cultivation conditions is an option to induce the production of desired biochemicals. Baer et al. (2016) for example, studied the effects of spectral light quality on biomass and phycobiliprotein productivities. Wu et al. (2015) reported the variation of pigment and chlorophyll composition and antioxidant activities under light stress. The effect of light and nitrogen availability on growth and carotenoid accumulation in *Scenedesmus* sp. was studied by Přibyl et al. (2016). Moreover, nutrients deficiency (Cakmak et al., 2012), CO_2 content of off-gas (Xia et al., 2013), pH (Eibl et al., 2014), lighting duration (Choi et al., 2015), temperature (Xin et al., 2011) and hydrodynamic shear stress (Song et al., 2014) have been all reported as triggers of lipid synthesis in microalgal cells.

Cultivation of microalgae can be carried out in either open or closed systems. Open systems such as raceways or tanks need less capital investment and labor, and have lower operation and maintenance costs compared to closed systems. Open systems are typically buried in the ground, to reduce the cost of construction and rely on sunlight to avoid the need for artificial lighting thereby reducing supplied energy requirements. The use of sunlight along with sparged-in industrial off-gas as a free source of CO_2, can both significantly reduce production costs. However, poor utilization rates of light and CO_2 in open ponds has been reported as drawbacks of open systems compared to closed systems (Amaro et al., 2012).

Open systems operate at shallow depths (15-30 cm) due to light attenuation with penetration depth (Kumar et al., 2015). These shallow depths result in large footprints and inefficient CO_2 transfer from sparged-in industrial off-gas due to short residence times. A deep, top-lit gas-lift bioreactor has been proposed to improve the CO_2 transfer rate by increasing the gas-liquid contact time as well as reducing the physical footprint of algal cultivation systems (Seyed Hosseini et al., 2016). Biomass productivity and lipid production for conversion into biodiesel was enhanced significantly due to light and hydrodynamic stresses. The use of a gas-lift system provided vertical circulation of algal cells from dark to light zones and good mixing, thereby avoiding the expense of additional artificial lighting. Microalgal cells convert light energy to chemical energy in the light zone and the chemical energy is further used for carbon fixation and biomass production. However, the optimal conditions for growing algae require only 20% of the system cycle be in the dark region and efficiency decreases significantly when this is above 50% (Janssen et al., 2000).

The surface of a top-lit culture receives the highest light intensity, which decreases exponentially with depth. As the culture gets more dense over time due to algal growth, light penetration into the culture is reduced, which inhibits cell growth (the shadow effect). One solution to increase light exposure and thereby be able to use deeper cultivation tanks is to use subsurface artificial lighting. This would, however, increase biomass production costs. To avoid the additional costs of installing, running and maintaining the sub-surface artificial lighting in a deep bioreactor, the use of a non-energy-consuming ‘light column’ in the center of the deep bioreactor is examined in this study. As microalgae cells are not present in the light column, the light can diffuse deeper

into the bioreactor without being blocked due to the shadow effect. The light column was filled with water to help increase the refraction of entering light and light utilization efficiency. Use of the water filled light column in the center of the top-lit gas-lift bioreactor increases the light-exposed surface area and light penetration into the deep culture as well as disturbing the mixing patterns.

In this study, the performance of a deep, top-lit gas-lift bioreactor coupled with a light column was evaluated for growth of *Scenedesmus dimorphus* and optimized through a two step, screening-optimization method for lipid production per unit area occupied by the bioreactor. In the factor screening step, the influence of operational parameters on lipid production were evaluated through application of a Plackett-Burman design. In the optimization step, the configuration of the significant operational parameters that maximize the lipid productivity was obtained through applying a response surface methodology. The result of the optimization process was then verified in the top-lit gas-lift bioreactor equipped with the light column.

6.2 Material and Methods

6.2.1 Microalgae strain and media

The green microalgae *Scenedesmus dimorphus* was obtained from the University of Texas, Austin collection (1,237 UTEX collection). Bold's Basal medium (BBM) (Andersen, 2005) was used for photoautotrophic cultivation. The stock culture was incubated at 25°C and 125 rpm. It was illuminated at 80 $\mu molm^{-2}s^{-1}$ on a photoperiod of 12 h light/12 h dark and fed with Bold's Basal growth medium every three weeks.

6.2.2 The bioreactors setup

The bioreactor used in this study was a concentric draft-tube, gas-lift column. A draft tube with an internal diameter of 0.13 m and height of 0.8 m, was secured in a 0.2 m-diameter outer column and placed 0.05 m above the sparger (Figure 6.1A). They were fabricated out of transparent Plexiglas with a wall thickness of 5 mm. Air mixed with carbon dioxide to simulate off-gas was sparged through a 0.10 m diameter ceramic sparger with a mean pore size of 15 μm (Refractron Technologies Corp., NY, USA). The bioreactor had side ports at 0.05 m and 0.5 m from the base for taking samples. The depth of the bioreactor was varied by changing the dispersion height (Δh), which is the distance from top of the draft tube to the surface of the culture. The flow rate was controlled using rotameters (Omega Engineering Ltd., QC, Canada).

As an open algal cultivation system, the bioreactor would be buried outside and light can not penetrate from the sides. In the laboratory setting, therefore, the outside of the bioreactor was covered with a layer of black plastic sheet on top of a white plastic sheet to block light entrance from the side. Light energy for the photosynthesis was provided only from the top of the bioreactor to simulate buried open systems on an industrial site, which is top lit by sunlight only. A 90 watt circular grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) was used to provide a light/dark photoperiod. The photosynthetic active radiation at the surface of the culture was kept around 1,000 $\mu molm^{-2}s^{-1}$ by changing the distance between the light source and culture surface at different depths.

The light column made of transparent Plexiglas with a 0.05 m diameter was inserted in the center of the draft tube. The light column was filled with water in order to increase the refraction of light. The presence of the light column in the center of the draft tube changed the mixing pattern and turbulence. In order to evaluate its effect on productivity, the results were compared to a bioreactor where the light column was covered with black plastic (dark column) to prevent light transmission while replicating the turbulence produced by the light column.

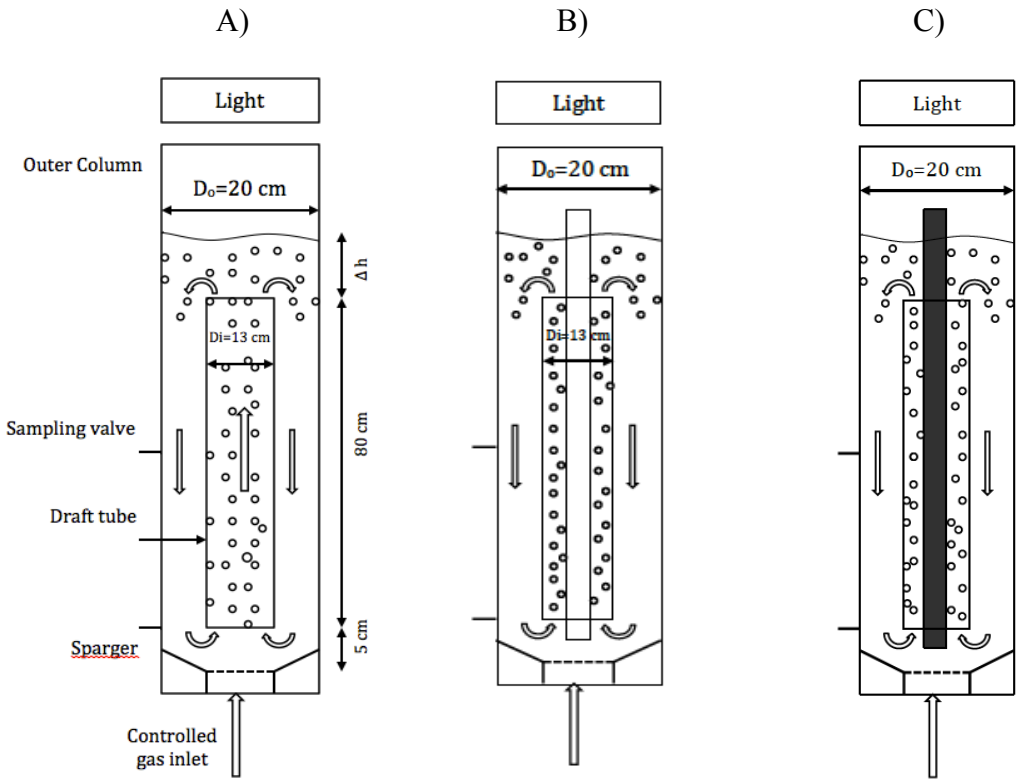


Figure 6.1: Schematic diagram of (A) top-lit gas–lift bioreactor, (B) top-lit gas–lift bioreactor equipped with a light column and (C) top-lit gas–lift bioreactor equipped with a dark column

The light intensity was measured with a light meter (LI-250 A, LI-COR Biosciences, NE, USA) equipped with a quantum sensor (LI-193SA, LI-COR Biosciences, NE, USA). The light intensity was measured at increments of known distances below the surface to identify the transition from the light zone to the dark zone. An intensity of 50 $\mu molm^{-2}s^{-1}$ was considered the minimum intensity for the light zone (Barbosa et al., 2003b) to estimate light fraction (ξ) as a ratio of the time algal cells spend in the light zone (t_l) over the mean circulation time (t_c).

$$\xi = \frac{t_l}{t_c} \quad (1)$$

The mean circulation time was calculated by measuring the time taken for a 5 mm colored tracer bead (Engineering Laboratories, NJ, USA) with the same density as water to circulate one cycle through an arbitrarily chosen horizontal reference plane in the bioreactor (Seyed Hosseini et al., 2015). Make up water was supplied every day to compensate for evaporative losses and experiments were carried out at 22±2°C.

6.2.3 Experimental design

The design of the experiment consisted of two steps. The first step, factor screening, employed a five-factor two-level Plackett-Burman (PB) design for evaluating the significance of independent factors on biomass and lipid productivities. Inserting the light column in the center of the gas-lift bioreactor changed the light propagation in terms of angles and penetration distance as well as the mixing pattern of gas bubbles in the riser and increased the turbulence and bubble-bursting rate. Therefore, the main effect and the interactions of factors involving the CO_2 and light absorption rates as main components

of the photosynthesis reaction should be evaluated. The factors affecting the CO_2 transfer rate and light utilization rate selected in this study include: use of light column (X_1), sparging rate (X_2), CO_2 concentration (X_3), depth (X_4) and lighting duration (X_5). An operational range for each factor was selected and the minimum and maximum levels were coded as -1 and +1. The light column was a categorical factor where -1 represented the use of the dark column and +1 represented the use of the light column filled with water in the center of the top-lit gas-lift bioreactor. The sparging rate was selected in the range of 4-14 $Lmin^{-1}$ to provide the optimum light fraction allowance of 50%-80% (Seyed Hosseini et al., 2015). The bioreactors were sparged with air (0.038%) and 6% CO_2–enriched air, to simulate the CO_2 content of a smelter's off-gas (Shang and Scott, 2011). The depth was evaluated in the range of 100 cm to 130 cm. For the lighting duration, a 16 h light / 8 h dark cycle was used to represent the peak day-light season and an 8 h light / 16 h dark cycle was used for the low day-light season labeled +1 and -1, respectively.

The screening step was conducted in two blocks of six bioreactors. Blocking was applied due to the limitation of experimental set-up to run at the same time. The experimental design and the obtained responses, including areal biomass productivity (P_a) and lipid production per unit area occupied by the bioreactor (P_{La}) are presented in Section 6.3.2. The results were analyzed statistically using Minitab-16 software. The significant factors, at a confidence level of 95% (p-value < 0.05) from regression analysis were further optimized at the second step by applying the response surface methodology (RSM).

The response surface method employed a five-level, three-factor, full factorial central composite design (CCD) with the three significant factors being: sparging rate (X_2),

depth (X_4), and lighting duration (X_5) (Table 6.1). In order to elicit more significant responses, the range over which the factors were changed was increased. The optimization phase consisted of 20 runs in three blocks, including 8 factorial (cubic points), 6 axial (star points), and 2 replicates of center points for each block. The obtained results were then fitted to a quadratic polynomial model to predict the response surfaces over the studied ranges of the factor variations as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (2)$$

where Y stands for the predicted response, β_0 is the independent coefficient, β_i is the linear coefficient associated to each independent factor (X_i), and β_{ii} and β_{ij} ($i \neq j$) are the coefficients for quadratic and interaction effects of X_i and X_j, respectively. The regression coefficients were determined by the method of least squares using Minitab software.

Table 6.1: Coded and actual levels of the factors designed through the CCD approach

Code	Factor	Coded levels				
		-1.633	-1	0	+1	+1.633
X_2	Sparging rate (L/min)	3	6	11	16	19
X_4	Depth (cm)	100	105	115	125	130
X_5	Lighting duration (h)	8	9.5	12	14.5	16

6.2.4 Analytical procedures

Microalgal growth was tracked by measuring the dry biomass concentration (C_b) through vacuum filtration of 100 ml of algal culture through glass microfiber filter paper (Grade 151, Ahlstrom Filtration LLC, PA, USA) followed by oven-drying over night at 60°C.

Areal biomass productivity (P_a), was calculated from the initial (C_{bi}) and final (C_{bf}) dry biomass concentrations over the exponential growth phase (Δt) as follows (Andersen, 2005):

$$P_a = \frac{1000\,(C_{bf} - C_{bi})V_t}{\Delta t.A_t} \quad (3)$$

where V_t is total volume of the bioreactor and A_t is area occupied by the bioreactor.

Lipid production per unit area occupied by the bioreactor was determined from the algal cell lipid content (% $g_{Lipid}/g_{dw,Biomass}$), and biomass concentration ($g_{dw}L^{-1}$). The lipid content of the microalgae was extracted through a modification of the method described by Folch et al. (1957). Algal biomass was harvested by centrifugation, frozen at -80°C and subsequently freeze-dried at -50°C under vacuum for 24 h. Freeze-dried biomass was then mixed with 1.2 mL of chloroform:methanol (2:1 v/v) in a centrifuge tube and sonicated for 30 minutes using a Sonic Dismembrator Model 500 (Fisher Scientific, Ottawa, Canada). The samples were centrifuged using an Allegra X-15R Centrifuge (Beckman, Palo Alto, CA) and the supernatant was removed into a weighed tube. Extraction was repeated in triplicate with the collected solvent layers being combined. The solvent was then evaporated in a vacuum drier and the mass of the remaining lipid determined gravimetrically (Seyed Hosseini et al., 2015). Areal lipid production (P_{La}) was then calculated as follows:

$$P_{La} = \frac{1000 C_b \theta_L V_t}{A_t} \quad (4)$$

where θ_L is the lipid content of microalgal cells.

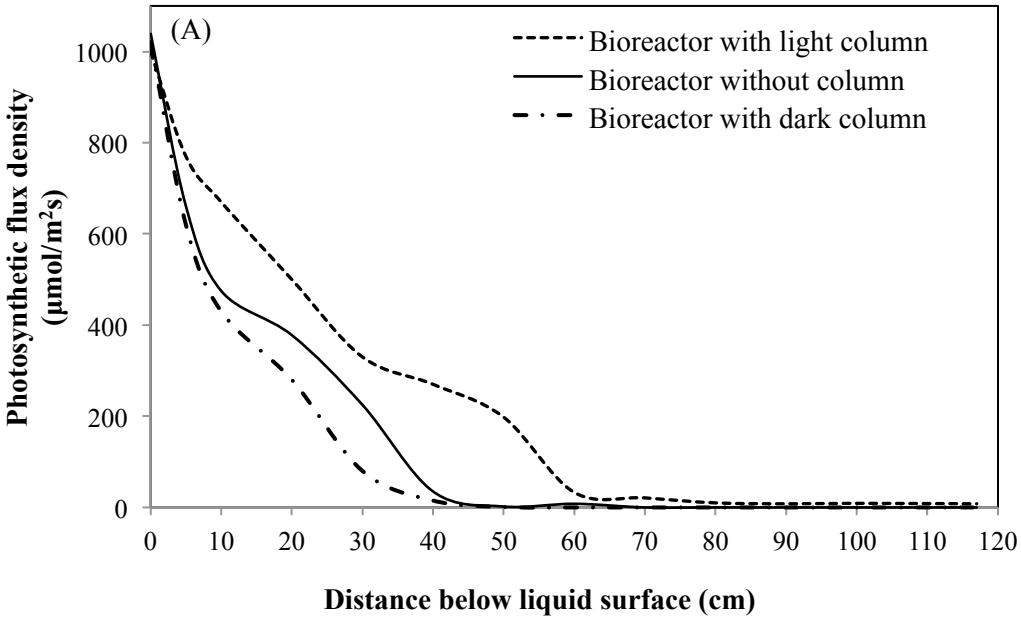
6.3 Results and discussion

6.3.1 Light column effectiveness

A comparative study was done with respect to the microalgal growth rate to evaluate the effectiveness of using a light column in a top-lit gas–lift bioreactor. The optimum operational configuration for *S. dimorphus* growth that maximized production of lipids suitable for conversion into the biodiesel was developed previously (Chapter Five). These optimal settings were used in this study with three top-lit 1.17 m deep bioreactors sparged with 6.4% CO_2-enriched air at a gas flow rate of 17.7 $Lmin^{-1}$. The three bioreactors were one without a light column, one with a water-filled light column inserted in the center of the draft tube and one with a dark column (same dimensions as the light column) inserted in the center of the draft tube (Figure 6.1).

Light intensity variations relative to bioreactor depth as determined by readings taken at 5 cm increments from the surface are shown in Figure 6.2A. The biomass density of 0.08 $g_{dw}L^{-1}$ and gas flow rate of 17.7 $Lmin^{-1}$ are similar in three bioreactors. As expected, the decrease in light intensity at increased depths due to the presence of algal cells (self-shading) and gas bubbles is greater in the bioreactor with the dark column. This can be explained by blocking the reflective surfaces and absorbing some of light waves by dark column. On the other hand, the light penetration is increased with using the water filled light column due to the enhanced light refraction.

The resulting variations in biomass concentration over three weeks for the three types of bioreactor are presented in Figure 6.2B.



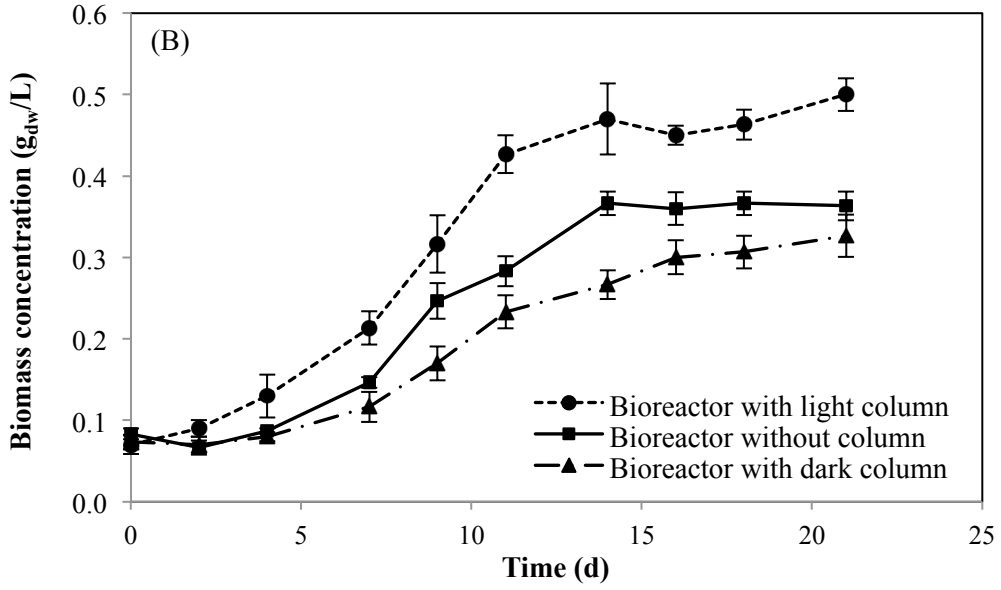


Figure 6.2: (A) Light intensity variation with depth; (B) Growth profile of *Scenedesmus* sp. in the top-lit gas-lift bioreactors; error bars show the standard error of triplicate experiments.

As can be seen, using the light column enhanced microalgal growth rate and increased biomass productivity by 33.6%. Comparing the growth profile of the culture coupled with the light column to the culture coupled with the dark column confirmed that this improvement in productivity is not the result of the change of mixing pattern and higher turbulence, but enhanced light utilization efficiency due to increased light penetration depth and higher light-exposed surface area. That is, the bioreactor with the light column provided a larger light zone that led to longer residence time of algal cells in the light zone and consequently absorbing more light energy, which was converted to the chemical energy required for algal growth. The dark column had a significant negative impact on algal growth rate. This is due to impeding the reflection of light waves as they traveled down the column and reduced light zone.

Utilizing this non-energy consuming method increased productivity without increasing energy consumption, which could play a critical role in the economics of biodiesel production.

6.3.2 Factor screening

Once the effectiveness of using the light column was confirmed, the impact of operational parameters that were affected by the increased light exposure on the biomass growth rate and lipid synthesis of microalgae was evaluated. The factors selected in the screening phase included the light column (X_1), sparging rate (X_2), CO_2 concentration (X_3), depth (X_4) and lighting duration (X_5).

Statistical analysis was applied on the responses of areal biomass productivity (P_a) and lipid production per unit area occupied by the bioreactor (P_{La}), achieved from a Plackett-

Burman (PB) design consisting of 12 experiments to evaluate the main effects of the tested factors. Table 6.2 presents the experimental design constructed by the PB approach and the obtained responses for P_a and P_{La}. By applying the quadratic least squares (QLS) method, the model coefficients were calculated and the root mean square error (RMSE) of the quadratic least squares analysis of each response was then determined.

By employing classical analysis on biomass productivity per unit area occupied by the bioreactor, all five factors: X_1 ($p = 0.004$), X_2 ($p = 0.025$), X_3 ($p = 0.000$), X_4 ($p = 0.024$) and X_5 ($p = 0.031$) were significant at a confidence level of 95%. This can be explained by the influence of depth and gas rising velocity on the CO_2 transfer rate to the algal cells as well as the impact of light on the CO_2 fixation rate during the photosynthesis process. Regarding the areal lipid production, similar results were obtained; X_1 ($p = 0.002$), X_2 ($p = 0.045$), X_3 ($p = 0.000$), X_4 ($p = 0.023$) and X_5 ($p = 0.006$) had significant effects on lipid synthesis. The significant impact of lighting and hydrodynamic conditions on the lipid accumulation in microalgal cells are in line with studies of Choi et al. (2015) and Song et al. (2014). The R^2 values were 96.6% and 98.2% for the areal biomass productivity and lipid production models respectively, representing a good accuracy for the predictions of the responses.

The application of the light column (X_1) and varying the CO_2 concentration (X_3) had the highest probabilities of positive influence on *Scenedesmus dimorphus* biomass and lipid formation. Therefore, X_1 and X_3 were kept fixed at their maximum levels and the impacts of sparging rate (X_2), depth (X_4) and lighting duration (X_5) evaluated in the optimization study.

Table 6.2: Plackett-Burman design of experiments and obtained responses to evaluate the main effect of operational factors

Run	Factors					Responses	
	X_1	X_2	X_3	X_4	X_5	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	Areal lipid production ($g_{Lipid}m^{-2}$)
1	-1	-1	-1	-1	-1	15.0	64.7
2	+1	-1	+1	+1	-1	35.6	168.5
3	-1	-1	+1	+1	+1	31.4	154.2
4	+1	+1	+1	-1	+1	43.6	190.7
5	+1	+1	-1	+1	-1	6.7	59.4
6	-1	+1	+1	-1	+1	30.2	162.3
7	-1	+1	+1	+1	-1	18.6	118.4
8	-1	-1	-1	+1	+1	10.8	84.6
9	+1	-1	+1	-1	-1	40.5	187.1
10	-1	+1	-1	-1	-1	4.2	62.6
11	+1	-1	-1	-1	+1	20.4	105.8
12	+1	+1	-1	+1	+1	16.3	92.4

6.3.3 Optimization

The response surface method as a five-level, three-factor central composite design (CCD) consisting of 20 experiments was applied to optimize the growth and lipid synthesis of microalgae *S. dimorphus* through evaluating the interactions of the significant parameters: the sparging rate (X_2), depth (X_4) and lighting duration (X_5). All bioreactors were equipped with a water-filled light column inserted in the center of the draft tube and sparged with 6% CO_2-enriched air.

The CCD design of experiments and the obtained results for areal biomass productivity (P_a) and lipid production per unit area occupied by the bioreactor (P_{La}) are provided in Table 6.3.

Table 6.3: Central composite design of experiment and obtained responses to determine the optimum biomass and lipid production of microalgae

Run	Factors Assignment			Responses	
	X_2	X_4	X_5	Areal biomass productivity ($g_{dw}m^{-2}day^{-1}$)	Areal lipid production ($g_{Lipid}m^{-2}$)
1	0	1.633	0	37.8	212.2
2	1.633	0	0	30.2	252.6
3	0	-1.633	0	37.0	230.1
4	0	0	-1.633	26.6	117.0
5	0	0	0	51.7	244.4
6	0	0	1.633	54.2	199.5
7	0	0	0	43.8	233.6
8	-1.633	0	0	33.6	77.2
9	-1	+1	-1	17.1	53.3
10	0	0	0	53.4	240.8
11	+1	-1	-1	23.4	166.7
12	+1	+1	+1	43.8	220.3
13	-1	-1	+1	49.0	183.4
14	0	0	0	55.0	250.8
15	-1	-1	-1	39.8	126.3
16	+1	+1	-1	32.2	133.2
17	-1	+1	+1	42.6	94.4
18	0	0	0	57.4	266.7
19	0	0	0	51.3	226.7
20	+1	-1	+1	31.8	188.1

The quadratic polynomial model (Eq. 2) was fitted on the results of experiments and the linear, quadratic and interaction coefficients of the model were calculated through the least squares analysis. The analysis of variance (ANOVA) of the obtained data is presented in Table 6.4.

Regarding the main effects of operational factors on areal biomass productivity and lipid production, only the sparging rate (X_2) and duration of lighting (X_5) were found significant (p-value < 0.05). Both factors exerted positive effects on the responses; meaning increasing the sparging rate and lighting duration are in favor of algal growth and lipid synthesis. Jacob-Lopes et al. (2009) also reported an increase in CO_2 fixation

and biomass production of microalgae when increasing the duration of the light period. Maroneze et al. (2016) stated an increase in lipid productivity when the photoperiod was changed from 12:12 to 24:0 (light:dark hours).

The depth (X_4) did not have significant impact on the biomass and lipid production of microalgal cells, whereas in a previous study (Chapter Five), the bioreactor depth did have a significant effect due to its influence on the gas-liquid contact time and CO_2 transfer rate. The outcomes from this study illustrate the beneficial impact of the light column in providing deeper light penetration. This indicates that when the light column is being used the depth of the bioreactor can be varied in the studied range without affecting the efficiency of the algal cultivation system.

Considering the interaction effects of operational factors, only the interaction of sparging rate and depth (X_2X_4) was significant for both P_a and P_{La} responses and exerting a positive effect with a *p*-value of 0.002 and 0.045, respectively. This is in agreement with a previous study (Chapter Five) and could be due to their compound impact on the residence time of the gas bubbles in the light and dark zones of the culture. The interaction of depth and sparging rate also has influence on the culture circulation frequency, or in other words the impact of the flashing light effect created by continual passing of algal cells between the light and dark zones. There are many studies on the effect of flashing light on the growth and lipid synthesis of microalgal cells. For example, Liu et al. (2014) and Xue et al. (2011) reported an increase in carbon fixation and specific growth rate of algal species with an intermittent illumination. Yoshioka et al. (2012) and He et al. (2015b) also reported an enhanced lipid yield due to the flashing light effect.

Table 6.4**:** Analysis of variance for response surface quadratic models

Factor	P_a ($g_{dw}m^{-2}day^{-1}$)		P_{La} ($g_{Lipid}m^{-2}$)	
	Regression coefficient	*p*-value	Regression coefficient	*p*-value
Regression coefficients including all factors and their second-order interactions				
Constant	**-105.9**	**0.020**	**-885.1**	**0.005**
Blocks	-1.549	0.339	11.56	0.256
X_2	**4.276**	**0.042**	**26.49**	**0.047**
X_4	0.666	0.499	0.125	0.984
X_5	**18.23**	**0.003**	**149.7**	**0.001**
X_2X_2	**-0.300**	**0.000**	**-1.442**	**0.001**
X_4X_4	**-0.054**	**0.001**	-0.149	0.053
X_5X_5	**-0.690**	**0.003**	**-6.166**	**0.000**
X_2X_4	**0.125**	**0.002**	**0.402**	**0.045**
X_2X_5	-0.147	0.203	0.103	0.883
X_4X_5	0.098	0.103	0.248	0.483
Regression coefficients including significant factors for reduced model				
Constant	-121.8	0.003	-834.1	0.000
X_2	2.512	0.099	26.79	0.010
X_4	1.836	0.039	-5.863	0.022
X_5	19.54	0.001	154.1	0.000
X_2X_2	-0.300	0.000	-1.399	0.000
X_4X_4	-0.054	0.001	N/A	N/A
X_5X_5	-0.690	0.003	-5.995	0.000
X_2X_4	0.125	0.001	0.402	0.058

- Bold values represent statistically significant results

The quadratic effects of two factors of X_2 and X_5 were also significant for both responses. In order to satisfy the model hierarchy requirement and avoid the lack of fit, the main effect of the depth (X_4) was also included in the model and generated a positive effect on both responses. Blocking due to the limitations of the experimental set-up to run at the same time did not have any significant effect on the responses. The probability values of both models were smaller than 1×10^{-4} and the lack of fits were not significant in the two models, indicating highly reliable model prediction.

Mathematical models for areal biomass productivity (P_a) and lipid production per unit area occupied by the bioreactor (P_{La}) are presented in equations (5) and (6). The Models are reduced to include only the significant factors.

$$P_a = -121.8 + 2.5\,X_2 + 1.8\,X_4 + 19.5\,X_5 - 0.3\,X_2^2 - 0.1\,X_4^2 - 0.7\,X_5^2 + 0.1\,X_2X_4 \quad (5)$$

$$P_{La} = -834.1 + 26.8\,X_2 - 5.9\,X_4 + 154.1\,X_5 - 1.4\,X_2^2 - 6.0\,X_5^2 + 0.4\,X_2X_4 \quad (6)$$

The coefficients of determination (R^2) of the predicting models were 91.3% and 87.7% for P_a and P_{La}, respectively. This means that the models could accurately predict 91% and 87% of the variability of the system.

The main effects of the operational factors and their interaction over the responses are illustrated in Figure 6.3. The third factor was kept constant at the medium level for all figures.

As can be seen in Figure 6.3A, since the interaction of sparging rate with depth has a significant effect on biomass production, the maximum biomass productivity per unit area occupied by the bioreactors is a critical value. Biomass production decreased with an increase in the sparging rate higher than a maximum value that is depth dependent due to the hydrodynamic stress. Barbosa et al. (2004) also reported a decrease in cell growth rate when increasing the gas flow rate, but an increase when increasing culture media depth. A similar observation is shown in Figure 6.3B for the interaction of lighting duration and depth on biomass productivity. As expected, increasing light exposure enhanced biomass productivity.

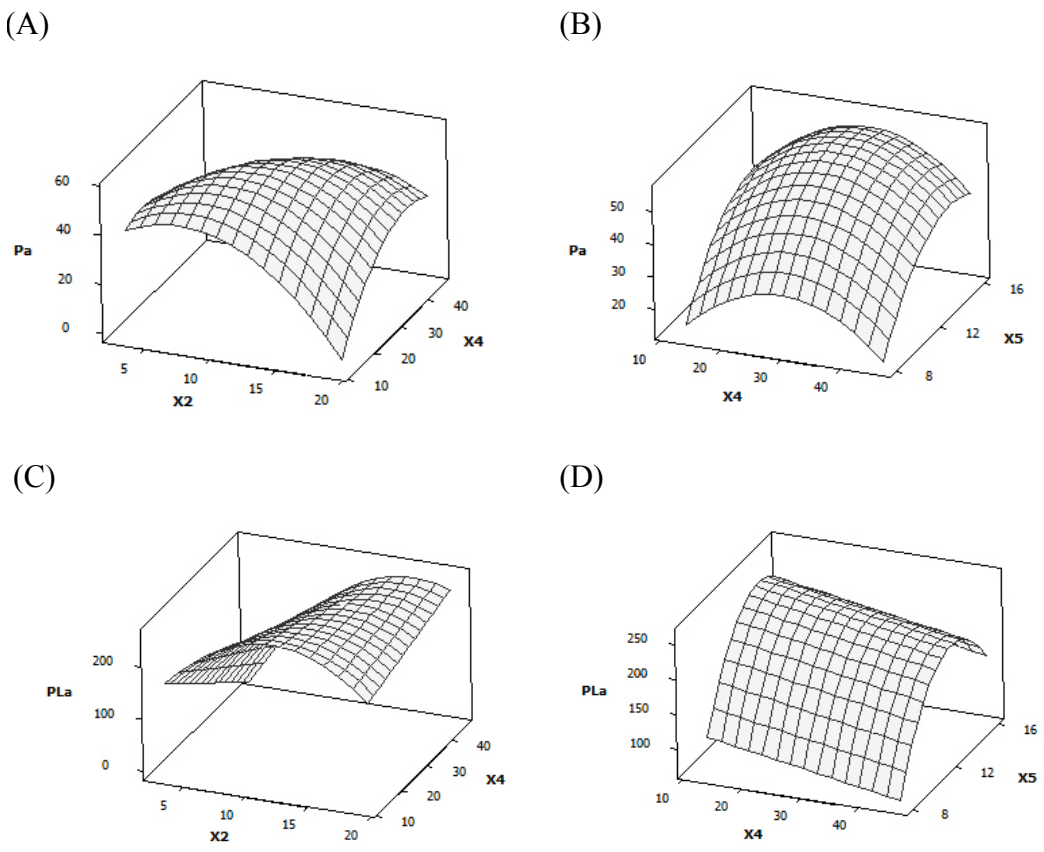


Figure 6.3: Response surfaces of the areal biomass productivity (P_a) and the lipid production per unit area (P_{La}) as a function of the sparging rate (X_2), depth (X_4) and lighting duration (X_5). The third factor was kept constant at its medium level for all figures.

Figure 6.3C demonstrates the interaction of sparging rate and depth on lipid production. The depth showed a linear effect on the areal lipid production. An increase in the sparging rate at the deeper culture resulted in an enhanced lipid production per unit area due to increased circulation frequency that resulted in hydrodynamic and light stresses. The impact of lighting duration and depth is demonstrated in Figure 6.3D. An increase in the light duration had a negative impact on the lipid production per unit area after a critical level. This result was in agreement with the study of Atta et al. (2013) that stated the lipid content decreased by increasing the photoperiod from 12 hours to a 16 hour light

period at the same light intensity. The decrease in lipid content at higher luminance exposure was considered due to higher chloroplastidial activity to prevent photochemical cell damage.

In order to maximize the lipid production per unit area and to minimize production costs, a desirability function (D) was applied to the operational factors in the study range. The criteria used for the desirability function and the optimum configuration of the tested factors to maximize the lipid production per unit area occupied by the bioreactor equipped with the light column sparged with 6% CO_2-enriched air are presented in Table 6.5.

By employing the desirability function, cultivation of *S. dimorphus* at a sparging rate of 13.7 $Lmin^{-1}$ and a depth of 102.5 cm under 12.5 h light/11.5 h dark photoperiod, would result in the maximum lipid production of 255.2 g_{Lipid} per square meter occupied by the bioreactor. In addition by setting the minimum biomass productivity criteria for the desirability function, energy requirements and processing costs such as harvesting, drying, transportation and lipid extraction to convert biomass into biodiesel would be reduced.

Table 6.5: The optimization of multiple responses requirements and suggested optimal configuration of factors

Factors and Responses	Optimization Criterion	Lower Limit	Upper Limit	Optimal Level
X_2 (L/min)	In range	3	19	13.7
X_4 (cm)	In range	100	130	102.5
X_5 (h)	In range	8	16	12.5
P_a ($g_{dw}m^{-2}day^{-1}$)	Minimize	17.1	57.4	31.2
P_{La} ($g_{Lipid}m^{-2}$)	Maximize	53.3	266.7	255.2
D (-)	-	-	-	0.98

6.3.4 Verification of optimization

Once the optimum configuration of operational factors was determined, the predicted responses were verified. *Scenedesmus* sp. was cultivated, therefore, in a 103 cm deep, bioreactor with and without a light column, sparged with 0.424 vvm CO_2-enriched air under a 12.5 h light/ 11.5 h dark photoperiod, as determined in the optimization step. The microalgal growth rate, lipid content and areal lipid production profiles of the cultures over three weeks are presented in Figure 6.4.

Biomass productivity of the bioreactor with the light column was 23.7% higher than that of the bioreactor without a light column as presented in Figure 6.4A. As can be seen in Figure 6.4B, the lipid content of algal cells in the bioreactor with the light column was 11% lower than that in the bioreactor without the light column. Atta et al. (2013) also reported a 13% decrease in lipid content of *C. vulgaris* in higher light exposure due to higher chloroplastidial activity of algal cells. The lipid production rate per unit area of 223.9 g_{Lipid}/m^2, however, was not affected by the decreased lipid content due to the higher biomass concentration. The areal lipid production in the bioreactor with the light column was 16% higher than that of the bioreactor without a light column.

Both experimental responses were in agreement with the predicted values obtained form the optimization models within ±10%, indicating accurate prediction by the models in the studied range.

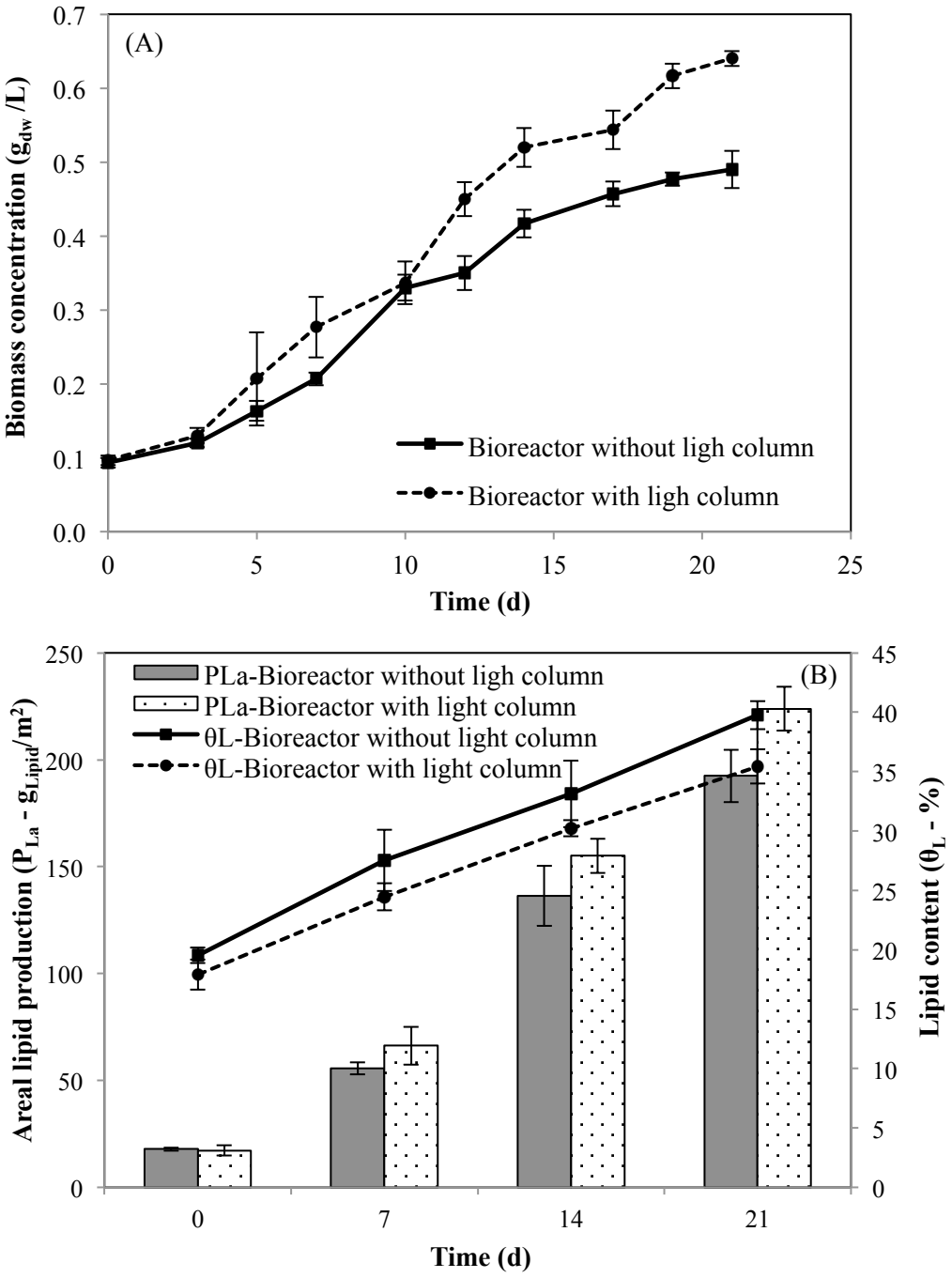


Figure 6.4: **(**A) Growth rate of *Scenedesmus* sp. in a top-lit gas-lift bioreactor with and without a light column; (B) Lipid production per unit area occupied by the bioreactors (Bar chart) and lipid content (Scatter chart); Error bars indicate the standard error of triplicate observations.

The areal biomass productivity and lipid production per unit area occupied by the bioreactors are summarized in Table 6.6. The maximum lipid produced in this bioreactor is 2,239 kg_{Lipid}/ha. By considering an average biodiesel density of 920 kg/m^3, the lipid volume is 2.43 m^3/ha, which would result in annual lipid production of 42.2 m^3/ha based on the batch productions. This result is comparable to that reported by Chisti (2007) for a raceway pond (42.6 m^3/ha) with significantly greater physical footprint.

Table 6.6: Predicted and experimental responses values for the tested bioreactors

Bioreactors	P_a ($g_{dw}m^{-2}day^{-1}$)		P_{La} ($g_{Lipid}m^{-2}$)	
	Predicted	Experimental	Predicted	Experimental
Bioreactor without light column	N/A	27.8	N/A	192.5
Bioreactor with light column	31.2	34.4	255.2	223.9

6.4 Conclusion

The top-lit gas-lift bioreactor for growing microalgae that can produce lipid for conversion into biodiesel has been previously developed as a promising option to reduce industrial off-gas CO_2 emissions with minimum physical footprint on industrial sites.

A modification of a top-lit gas-lift bioreactor sparged with CO_2-enriched air to simulate industrial off-gas to increase the biomass and lipid productivities was proposed in this study. A transparent column filled with water (light column) inserted into the center of the draft tube of the gas-lift bioreactor increased light utilization efficiency without an increase in energy supply costs. The result was an increase in microalgal biomass productivity by 28% on average. The areal lipid production was also increased by 16% by using the light column.

The proposed cultivation system can be coupled with any industrial sectors dependent upon fossil fuels in order to improve sustainability though CO_2 mitigation and biodiesel production.

Chapter Seven: Conclusion

The feasibility and efficiency of using a gas-lift circulating system to enable deeper open cultivation systems has been studied. The novel approach used was to use a one-meter deep cylindrical bioreactor with a minimum depth of one meter, equipped with a gas-lift circulating system and fed with CO_2-enriched air that emulates feeding with industrial off-gas. The bioreactor was top-lit in order to simulate outdoor sunlight conditions. The achieved goal of the bioreactor configuration presented was to increase operational depths without the additional cost of internal, sub-surface artificial lighting.

In Chapter Three, microalgae cultivation in the one-meter deep top-lit gas-lift bioreactor showed comparable volumetric biomass productivity (0.06 $g_{dw}L^{-1}day^{-1}$) to traditional large-scale open systems. However, the high biomass productivity per unit area occupied by the gas-lift bioreactor was three-times higher than that reported for traditional raceways, which showed there was enhanced CO_2 sequestration. This would significantly help in reducing the physical footprint of large-scale microalgal production plant and make it easier to locate them close to industrial off-gas sources. Furthermore, microalgal cell lipid content was increased by 27% due to a low pH stressing environment caused by the high CO_2 content of sparged gas.

In Chapter Four, two common gas-liquid contacting systems (gas-lift and bubble column bioreactors) were evaluated and compared for microalgal biomass and lipid production. The bubble column presented 12% greater biomass production than the gas-lift bioreactor. This is due to the increased velocity of the bubbles in the smaller diameter draft tube of the gas-lift bioreactor resulting in shorter gas residence times. However, a

higher volumetric lipid production was obtained in the gas-lift bioreactor, which was due to continual vertical circulation of microalgae between the dark and light zones, known as the flashing light effect. The energy requirement analysis and hydrodynamic model developed in this chapter can be readily adapted to meet scalability requirements.

Lipid produced from a microalgae cultivation facility is the primary feedstock for biodiesel production. As mentioned in Chapter Two, operational and environmental factors can be modified to trigger microalgal cells to synthesize more lipids. Therefore, as discussed in Chapter Five, various operational parameters that are known to have influence on biomass and lipid production were screened and optimizations made to ensure the highest output of suitable lipids for conversion into biodiesel. Among operational parameters selected for study, the initial biomass density and nutrient concentration did not have significant effects on biomass and lipid production. Consequently, by minimizing the nutrient concentration for microalgal growth in the lipid production process, significant environmental and economic impacts could be obtained.

Mathematical models to predict areal biomass and lipid production were developed through response surface methodology, which was then used to estimate the optimal combination of operational parameters for maximization of lipid productivity. Operated at the optimal settings, the top-lit gas-lift bioreactor resulted in a ±10% agreement with the predicted results.

In Chapter Six, the additional benefits from a novel approach that uses a non-energy consuming light column in conjunction with the top-lit gas-lift bioreactor were demonstrated. The biomass production per unit area occupied by the bioreactor equipped

with the light column was increased by 33% along with a 16% increase in areal lipid productivity. This indicated enhanced light utilization efficiency in the deep cultivation systems without any increase in energy usage.

The use of the light column did result in a decrease in the lipid content of microalgal cells, possibly due to higher chloroplastidial activity. This did not, however, adversely affect the overall lipid production due to a substantial increase in the biomass concentration. The operational parameters influencing the lipid synthesis in the proposed configuration were also screened and optimized to reach maximum lipid production, while keeping the biomass production at a minimum. By producing the minimum amount of biomass with the maximum lipid output, the downstream process costs of harvesting, drying and lipid extraction would be lower, which would considerably improve the economics of biodiesel production.

Provisions for reducing the outdoor contamination of deep top-lit gas-lift bioreactors with invasive organisms that are competitors (non-target algae species) or predators (grazers) should be considered. For example, by using a culture medium with a higher salinity concentration or covering the bioreactors with a transparent material, contamination can be reduced. This would also help in reducing the loss of CO_2 and evaporative water. Depending on the source of the industrial off-gas, the presence and toxicity levels of inhibitory substances such as SO_x and NO_x is another limitation that should be addressed when using off-gas CO_2 as the source of carbon for photosynthesis. Selecting low-pH tolerant species of microalgae or using desulfurization methods could be a solution should this issue arise.

The proposed configuration in this study is easily scalable and can be applied to larger scale, on-site production facilities. This could open up new possibilities in combating the energy crisis and approaching the goal of becoming carbon-neutral through production of biofuels such as biodiesel as well as mitigation of CO_2 content in industrial off-gases.

7.1 Future direction

There are several directions in which this research can be continued, in particular moving to a pilot plant that can mimic the likely configuration of tanks located on an industrial site. Research to be done includes, but is not limited to:

- Evaluating the impact of increasing the overall depth beyond that studied in this work, including the height of the draft tube, the dispersion height, and the bioreactor diameter on areal biomass and lipid productivities – to determine if areal productivity can be further increased through the use of deeper gas-lift systems.
- Translating laboratory results into a pilot plant to verify model parameters and evaluate mixing patterns and effective light distribution on microalgae productivity – to study the impact of multiple draft-tubes co-located in a single tank.
- Manipulating operational and environmental parameters in the pilot plant to induce lipid synthesis under stress conditions including modifying pH, temperature, and nutrient concentrations – to determine if observations in the laboratory can be duplicated at a larger scale.
- Studying the impact of contaminations such as invasive organisms (competitors or predators) and toxic substances such as SO_x and NO_x contained in industrial off-

gases – a crucial study to ensure that the use of industrial off-gas and open tanks located on an industrial site can function long-term.

Bibliography

Abu-Ghosh, S., Fixler, D., Dubinsky, Z., Iluz, D., 2016. Flashing light in microalgae biotechnology. Bioresour. Technol. 203, 357–363. doi:10.1016/j.biortech.2015.12.057

Alaswad, A., Dassisti, M., Prescott, T., Olabi, A.G., 2015. Technologies and developments of third generation biofuel production. Renew. Sustain. Energy Rev. 51, 1446–1460.

Aléman-Nava, G.S., Muylaert, K., Cuellar Bermudez, S.P., Depraetere, O., Rittmann, B., Parra-Saldívar, R., Vandamme, D., 2017. Two-stage cultivation of Nannochloropsis oculata for lipid production using reversible alkaline flocculation. Bioresour. Technol. 226, 18–23. doi:10.1016/j.biortech.2016.11.121

Alharthi, K., Christianto, Y., Aguiar, A., Stickland, A.D., Stevens, G.W., Kentish, S.E., 2016. Impact of Fly Ash on the Membrane Performance in Postcombustion Carbon Capture Applications. Ind. Eng. Chem. Res. 55, 4711–4719. doi:10.1021/acs.iecr.6b00312

Al-Shayji, Y.A., Puskas, K., Al-Daher, R., Esen, I.I., 1994. Production and separation of algae in a high-rate ponds system. Environ. Int. 20, 541–550. doi:10.1016/0160-4120(94)90202-X

Amaro, H.M., Macedo, Â.C., Malcata, F.X., 2012. Microalgae: An alternative as sustainable source of biofuels? Energy, Integration and Energy System Engineering, European Symposium on Computer-Aided Process Engineering 2011 44, 158–166. doi:10.1016/j.energy.2012.05.006

Andersen, R.A., 2005. Algal culturing techniques. Elsevier Academic Press, Burlington, MA.

Anderson, T.R., Hawkins, E., Jones, P.D., 2016. CO_2, the greenhouse effect and global warming: from the pioneering work of Arrhenius and Callendar to today's Earth System Models. Endeavour 40, 178–187. doi:10.1016/j.endeavour.2016.07.002

Ansari, F.A., Singh, P., Guldhe, A., Bux, F., 2017. Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal Res. 21, 169–177. doi:10.1016/j.algal.2016.11.015

Asulabh, K.S., Supriya, G., Ramachandra, T.V., 2012. Effect of salinity concentrations on growth rate and lipid concentration in Microcystis sp., Chlorococcum sp. and Chaetoceros sp, in: National Conference on Conservation and Management of Wetland Ecosystems. School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala.

Atta, M., Idris, A., Bukhari, A., Wahidin, S., 2013. Intensity of blue LED light: A potential stimulus for biomass and lipid content in fresh water microalgae Chlorella vulgaris. Bioresour. Technol. 148, 373–378. doi:10.1016/j.biortech.2013.08.162

Badger, M.R., Price, G.D., 1994. The Role of Carbonic Anhydrase in Photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 369–392. doi:10.1146/annurev.pp.45.060194.002101

Baer, S., Heining, M., Schwerna, P., Buchholz, R., Hübner, H., 2016. Optimization of spectral light quality for growth and product formation in different microalgae using a continuous photobioreactor. Algal Res. 14, 109–115. doi:10.1016/j.algal.2016.01.011

Baicha, Z., Salar-García, M.J., Ortiz-Martínez, V.M., Hernández-Fernández, F.J., de los Ríos, A.P., Labjar, N., Lotfi, E., Elmahi, M., 2016. A critical review on microalgae as an alternative source for bioenergy production: A promising low cost substrate for microbial fuel cells. Fuel Process. Technol. 154, 104–116. doi:10.1016/j.fuproc.2016.08.017

Bao, Y., Liu, M., Wu, X., Cong, W., Ning, Z., 2012. In situ carbon supplementation in large-scale cultivations of Spirulina platensis in open raceway pond. Biotechnol. Bioprocess Eng. 17, 93–99. doi:10.1007/s12257-011-0319-9

Barbosa, M.J., Albrecht, M., Wijffels, R.H., 2003a. Hydrodynamic stress and lethal events in sparged microalgae cultures. Biotechnol. Bioeng. 83, 112–120. doi:10.1002/bit.10657

Barbosa, M.J., Hadiyanto, Wijffels, R.H., 2004. Overcoming shear stress of microalgae cultures in sparged photobioreactors. Biotechnol. Bioeng. 85, 78–85. doi:10.1002/bit.10862

Barbosa, M.J., Janssen, M., Ham, N., Tramper, J., Wijffels, R.H., 2003b. Microalgae cultivation in air-lift reactors: Modeling biomass yield and growth rate as a function of mixing frequency. Biotechnol. Bioeng. 82, 170–179. doi:10.1002/bit.10563

Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: A review. Renew. Sustain. Energy Rev. 41, 1489–1500. doi:10.1016/j.rser.2014.09.037

Bartley, M.L., Boeing, W.J., Corcoran, A.A., Holguin, F.O., Schaub, T., 2013. Effects of salinity on growth and lipid accumulation of biofuel microalga Nannochloropsis salina and invading organisms. Biomass Bioenergy 54, 83–88. doi:10.1016/j.biombioe.2013.03.026

Bartley, M.L., Boeing, W.J., Dungan, B.N., Holguin, F.O., Schaub, T., 2014. pH effects on growth and lipid accumulation of the biofuel microalgae Nannochloropsis salina and invading organisms. J. Appl. Phycol. 26, 1431–1437. doi:10.1007/s10811-013-0177-2

Belmabkhout, Y., Guillerm, V., Eddaoudi, M., 2016. Low concentration CO_2 capture using physical adsorbents: Are metal–organic frameworks becoming the new benchmark materials? Chem. Eng. J. 296, 386–397. doi:10.1016/j.cej.2016.03.124

Ben-Mansour, R., Habib, M.A., Bamidele, O.E., Basha, M., Qasem, N.A.A., Peedikakkal, A., Laoui, T., Ali, M., 2016. Carbon capture by physical adsorption: Materials, experimental investigations and numerical modeling and simulations – A review. Appl. Energy 161, 225–255. doi:10.1016/j.apenergy.2015.10.011

Bennett, M.C., Turn, S.Q., Chan, W.Y., 2014. A methodology to assess open pond, phototrophic, algae production potential: A Hawaii case study. Biomass Bioenergy 66, 168–175. doi:10.1016/j.biombioe.2014.03.016

Bhola, V., Swalaha, F., Ranjith Kumar, R., Singh, M., Bux, F., 2014. Overview of the potential of microalgae for CO_2 sequestration. Int. J. Environ. Sci. Technol. 11, 2103–2118. doi:10.1007/s13762-013-0487-6

Bielicki, J.M., Pollak, M.F., Deng, H., Wilson, E.J., Fitts, J.P., Peters, C.A., 2016. The Leakage Risk Monetization Model for Geologic CO_2 Storage. Environ. Sci. Technol. 50, 4923–4931. doi:10.1021/acs.est.5b05329

Bilanovic, D., Holland, M., Armon, R., 2012. Microalgal CO_2 sequestering – Modeling microalgae production costs. Energy Convers. Manag. 58, 104–109. doi:10.1016/j.enconman.2012.01.007

Borowitzka, M.A., 2013a. High-value products from microalgae—their development and commercialisation. J. Appl. Phycol. 25, 743–756. doi:10.1007/s10811-013-9983-9

Borowitzka, M.A., 2013b. Energy from Microalgae: A Short History, in: Borowitzka, M.A., Moheimani, N.R. (Eds.), Algae for Biofuels and Energy, Developments in Applied Phycology. Springer Netherlands, pp. 1–15. doi:10.1007/978-94-007-5479-9_1

Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J. Biotechnol. 70, 313–321. doi:10.1016/S0168-1656(99)00083-8

Bounaceur, R., Lape, N., Roizard, D., Vallieres, C., Favre, E., 2006. Membrane processes for post-combustion carbon dioxide capture: A parametric study. Energy 31, 2556–2570. doi:10.1016/j.energy.2005.10.038

Brindley, C., Jiménez-Ruíz, N., Acién, F.G., Fernández-Sevilla, J.M., 2016. Light regime optimization in photobioreactors using a dynamic photosynthesis model. Algal Res. 16, 399–408. doi:10.1016/j.algal.2016.03.033

Budzianowski, W.M., 2017. Useful Mechanisms, Energy Efficiency Benefits, and Challenges of Emerging Innovative Advanced Solvent Based Capture Processes, in: Budzianowski, W.M. (Ed.), Energy Efficient Solvents for CO_2 Capture by Gas-Liquid Absorption, Green Energy and Technology. Springer International Publishing, pp. 69–98. doi:10.1007/978-3-319-47262-1_4

Cakmak, T., Angun, P., Demiray, Y.E., Ozkan, A.D., Elibol, Z., Tekinay, T., 2012. Differential effects of nitrogen and sulfur deprivation on growth and biodiesel feedstock production of Chlamydomonas reinhardtii. Biotechnol. Bioeng. 109, 1947–1957. doi:10.1002/bit.24474

Camacho-Rodríguez, J., Cerón-García, M.C., Fernández-Sevilla, J.M., Molina-Grima, E., 2015. Genetic algorithm for the medium optimization of the microalga Nannochloropsis gaditana cultured to aquaculture. Bioresour. Technol. 177, 102–109. doi:10.1016/j.biortech.2014.11.057

Cao, J., Yuan, H., Li, B., Yang, J., 2014. Significance evaluation of the effects of environmental factors on the lipid accumulation of Chlorella minutissima UTEX 2341 under low-nutrition heterotrophic condition. Bioresour. Technol. 152, 177–184. doi:10.1016/j.biortech.2013.10.084

Cerri, M.O., Baldacin, J.C., Cruz, A.J.G., Hokka, C.O., Badino, A.C., 2010. Prediction of mean bubble size in pneumatic reactors. Biochem. Eng. J., Special Section: CHEMPOR2008 - Integration of Life Sciences and Engineering 53, 12–17. doi:10.1016/j.bej.2009.03.009

Cheah, W.Y., Ling, T.C., Juan, J.C., Lee, D.-J., Chang, J.-S., Show, P.L., 2016. Biorefineries of carbon dioxide: From carbon capture and storage (CCS) to bioenergies production. Bioresour. Technol. 215, 346–356. doi:10.1016/j.biortech.2016.04.019

Cheah, W.Y., Show, P.L., Chang, J.-S., Ling, T.C., Juan, J.C., 2015. Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. Bioresour. Technol. 184, 190–201. doi:10.1016/j.biortech.2014.11.026

Cheah, Y.E., Zimont, A.J., Lunka, S.K., Albers, S.C., Park, S.J., Reardon, K.F., Peebles, C.A.M., 2015. Diel light:dark cycles significantly reduce FFA accumulation in FFA producing mutants of Synechocystis sp. PCC 6803 compared to continuous light. Algal Res. 12, 487–496. doi:10.1016/j.algal.2015.10.014

Cheirsilp, B., Torpee, S., 2012. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. Bioresour. Technol. 110, 510–516. doi:10.1016/j.biortech.2012.01.125

Chen, G.-Q., Jiang, Y., Chen, F., 2008. Variation of lipid class composition in Nitzschia laevis as a response to growth temperature change. Food Chem. 109, 88–94. doi:10.1016/j.foodchem.2007.12.022

Chen, Y., Tang, X., Kapoore, R.V., Xu, C., Vaidyanathan, S., 2015. Influence of nutrient status on the accumulation of biomass and lipid in Nannochloropsis salina and Dunaliella salina. Energy Convers. Manag. 106, 61–72. doi:10.1016/j.enconman.2015.09.025

Cheng, J., Yang, Z., Ye, Q., Zhou, J., Cen, K., 2016. Improving CO_2 fixation with microalgae by bubble breakage in raceway ponds with up–down chute baffles. Bioresour. Technol. 201, 174–181. doi:10.1016/j.biortech.2015.11.044

Chiaramonti, D., Prussi, M., Casini, D., Tredici, M.R., Rodolfi, L., Bassi, N., Zittelli, G.C., Bondioli, P., 2013. Review of energy balance in raceway ponds for microalgae cultivation: Re-thinking a traditional system is possible. Appl. Energy, Special Issue on Advances in sustainable biofuel production and use - XIX International Symposium on Alcohol Fuels - ISAF 102, 101–111. doi:10.1016/j.apenergy.2012.07.040

Chinnasamy, S., Bhatnagar, A., Claxton, R., Das, K.C., 2010. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresour. Technol. 101, 6751–6760. doi:10.1016/j.biortech.2010.03.094

Chisti, Y., 2013. Raceways-based Production of Algal Crude Oil. Green 3, 195–216. doi:10.1515/green-2013-0018

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends Biotechnol. 26, 126–131. doi:10.1016/j.tibtech.2007.12.002

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306. doi:10.1016/j.biotechadv.2007.02.001

Chisti, Y., 1989. Airlift bioreactor. Elsevier Science Ltd, USA.

Chiu, S.-Y., Kao, C.-Y., Tsai, M.-T., Ong, S.-C., Chen, C.-H., Lin, C.-S., 2009. Lipid accumulation and CO_2 utilization of Nannochloropsis oculata in response to CO_2 aeration. Bioresour. Technol. 100, 833–838. doi:10.1016/j.biortech.2008.06.061

Choi, Y.-K., Kumaran, R.S., Jeon, H.J., Song, H.-J., Yang, Y.-H., Lee, S.H., Song, K.-G., Kim, K.J., Singh, V., Kim, H.J., 2015. LED light stress induced biomass and fatty acid production in microalgal biosystem, Acutodesmus obliquus. Spectrochim. Acta. A. Mol. Biomol. Spectrosc. 145, 245–253. doi:10.1016/j.saa.2015.03.035

Chokshi, K., Pancha, I., Trivedi, K., George, B., Maurya, R., Ghosh, A., Mishra, S., 2015. Biofuel potential of the newly isolated microalgae Acutodesmus dimorphus under temperature induced oxidative stress conditions. Bioresour. Technol. 180, 162–171. doi:10.1016/j.biortech.2014.12.102

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol. Adv. 29, 686–702. doi:10.1016/j.biotechadv.2011.05.015

Combe, C., Hartmann, P., Rabouille, S., Talec, A., Bernard, O., Sciandra, A., 2015. Long-term adaptive response to high-frequency light signals in the unicellular photosynthetic eukaryote Dunaliella salina. Biotechnol. Bioeng. 112, 1111–1121. doi:10.1002/bit.25526

Contreras, A., Chisti, Y., Molina, E., 1998. A reassesment of relationship between riser and downcomer gas holdups in airlift reactors. Chem. Eng. Sci. 53, 4151–4154. doi:10.1016/S0009-2509(98)00230-9

Creamer, A.E., Gao, B., Wang, S., 2016. Carbon dioxide capture using various metal oxyhydroxide–biochar composites. Chem. Eng. J. 283, 826–832. doi:10.1016/j.cej.2015.08.037

Dai, Z., Viswanathan, H., Middleton, R., Pan, F., Ampomah, W., Yang, C., Jia, W., Xiao, T., Lee, S.-Y., McPherson, B., Balch, R., Grigg, R., White, M., 2016. CO_2 Accounting and Risk Analysis for CO_2 Sequestration at Enhanced Oil Recovery Sites. Environ. Sci. Technol. 50, 7546–7554. doi:10.1021/acs.est.6b01744

Daroch, M., Geng, S., Wang, G., 2013. Recent advances in liquid biofuel production from algal feedstocks. Appl. Energy, Special Issue on Advances in sustainable biofuel production and use - XIX International Symposium on Alcohol Fuels - ISAF 102, 1371–1381. doi:10.1016/j.apenergy.2012.07.031

Das, P., Lei, W., Aziz, S.S., Obbard, J.P., 2011. Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. Bioresour. Technol. 102, 3883–3887. doi:10.1016/j.biortech.2010.11.102

de Godos, I., Mendoza, J.L., Acién, F.G., Molina, E., Banks, C.J., Heaven, S., Rogalla, F., 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. Bioresour. Technol. 153, 307–314. doi:10.1016/j.biortech.2013.11.087

De Silva, G.P.D., Ranjith, P.G., Perera, M.S.A., 2015. Geochemical aspects of CO_2 sequestration in deep saline aquifers: A review. Fuel 155, 128–143. doi:10.1016/j.fuel.2015.03.045

Demirbas, A., 2010. Use of algae as biofuel sources. Energy Convers. Manag. 51, 2738–2749. doi:10.1016/j.enconman.2010.06.010

Demirbas, A., Fatih Demirbas, M., 2011. Importance of algae oil as a source of biodiesel. Energy Convers. Manag. 52, 163–170. doi:10.1016/j.enconman.2010.06.055

Dhingra, S., Bhushan, G., Dubey, K.K., 2013. Development of a combined approach for improvement and optimization of karanja biodiesel using response surface methodology and genetic algorithm. Front. Energy 7, 495–505. doi:10.1007/s11708-013-0267-5

Doan, Y.T.T., Obbard, J.P., 2015. Two-stage cultivation of a Nannochloropsis mutant for biodiesel feedstock. J. Appl. Phycol. 27, 2203–2208. doi:10.1007/s10811-014-0490-4

Doucha, J., Straka, F., Lívanský, K., 2005. Utilization of flue gas for cultivation of microalgae Chlorella sp.) in an outdoor open thin-layer photobioreactor. J. Appl. Phycol. 17, 403–412. doi:10.1007/s10811-005-8701-7

Du, H., Lin, J., Zuercher, C., 2012. Higher efficiency of CO_2 injection into seawater by a venturi than a conventional diffuser system. Bioresour. Technol. 107, 131–134. doi:10.1016/j.biortech.2011.12.060

Eibl, J.K., Corcoran, J.D., Senhorinho, G.N.A., Zhang, K., Hosseini, N., Marsden, J., Laamanen, C.A., Scott, J.A., Ross, G.M., 2014. Bioprospecting for acidophilic lipid-rich green microalgae isolated from abandoned mine site water bodies. AMB Express 4, 7. doi:10.1186/2191-0855-4-7

Ertit Taştan, B., Duygu, E., İlbaş, M., Dönmez, G., 2016. Enhancement of microalgal biomass production and dissolved inorganic C fixation from actual coal flue gas by exogenous salicylic acid and 1-triacontanol growth promoters. Energy 103, 598–604. doi:10.1016/j.energy.2016.03.020

Esakkimuthu, S., Krishnamurthy, V., Govindarajan, R., Swaminathan, K., 2016. Augmentation and starvation of calcium, magnesium, phosphate on lipid production of Scenedesmus obliquus. Biomass Bioenergy 88, 126–134. doi:10.1016/j.biombioe.2016.03.019

Feng, P., Deng, Z., Fan, L., Hu, Z., 2012. Lipid accumulation and growth characteristics of Chlorella zofingiensis under different nitrate and phosphate concentrations. J. Biosci. Bioeng. 114, 405–410. doi:10.1016/j.jbiosc.2012.05.007

Folch, J., Lees, M., Sloane STanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Gardner, R.D., Cooksey, K.E., Mus, F., Macur, R., Moll, K., Eustance, E., Carlson, R.P., Gerlach, R., Fields, M.W., Peyton, B.M., 2012. Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte Scenedesmus sp. and the diatom Phaeodactylum tricornutum. J. Appl. Phycol. 24, 1311–1320. doi:10.1007/s10811-011-9782-0

Geyer, F., Schönecker, C., Butt, H.-J., Vollmer, D., 2016. Enhancing CO_2 Capture using Robust Superomniphobic Membranes. Adv. Mater. n/a-n/a. doi:10.1002/adma.201603524

Giordano, P.C., Beccaria, A.J., Goicoechea, H.C., 2014. Rational design of a culture medium for the intensification of lipid storage in Chlorella sp. Performance evaluation in air-lift bioreactor. Bioresour. Technol. 158, 269–277. doi:10.1016/j.biortech.2014.02.037

Goeppert, A., Czaun, M., Surya Prakash, G.K., Olah, G.A., 2012. Air as the renewable carbon source of the future: an overview of CO_2 capture from the atmosphere. Energy Environ. Sci. 5, 7833–7853. doi:10.1039/c2ee21586a

Goli, A., Shamiri, A., Talaiekhozani, A., Eshtiaghi, N., Aghamohammadi, N., Aroua, M.K., 2016. An overview of biological processes and their potential for CO_2 capture. J. Environ. Manage. 183, 41–58. doi:10.1016/j.jenvman.2016.08.054

González-López, C.V., Acién Fernández, F.G., Fernández-Sevilla, J.M., Sánchez Fernández, J.F., Molina Grima, E., 2012. Development of a process for efficient use of CO_2 from flue gases in the production of photosynthetic microorganisms. Biotechnol. Bioeng. 109, 1637–1650. doi:10.1002/bit.24446

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2010. Placing microalgae on the biofuels priority list: a review of the technological challenges. J. R. Soc. Interface 7, 703–726. doi:10.1098/rsif.2009.0322

Griffiths, M.J., Harrison, S.T.L., 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J. Appl. Phycol. 21, 493–507. doi:10.1007/s10811-008-9392-7

Han, F., Pei, H., Hu, W., Song, M., Ma, G., Pei, R., 2015. Optimization and lipid production enhancement of microalgae culture by efficiently changing the conditions along with the growth-state. Energy Convers. Manag. 90, 315–322. doi:10.1016/j.enconman.2014.11.032

Handler, R.M., Canter, C.E., Kalnes, T.N., Lupton, F.S., Kholiqov, O., Shonnard, D.R., Blowers, P., 2012. Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts. Algal Res. 1, 83–92. doi:10.1016/j.algal.2012.02.003

Hanson, D.T., 2016. Breaking the rules of Rubisco catalysis. J. Exp. Bot. 67, 3180–3182. doi:10.1093/jxb/erw197

Hasan, M.M.F., Boukouvala, F., First, E.L., Floudas, C.A., 2014. Nationwide, Regional, and Statewide CO_2 Capture, Utilization, and Sequestration Supply Chain Network Optimization. Ind. Eng. Chem. Res. 53, 7489–7506. doi:10.1021/ie402931c

He, Q., Yang, H., Wu, L., Hu, C., 2015a. Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae. Bioresour. Technol. 191, 219–228. doi:10.1016/j.biortech.2015.05.021

He, Q., Yang, H., Xu, L., Xia, L., Hu, C., 2015b. Sufficient utilization of natural fluctuating light intensity is an effective approach of promoting lipid productivity in oleaginous microalgal cultivation outdoors. Bioresour. Technol. 180, 79–87. doi:10.1016/j.biortech.2014.12.088

Heredia-Arroyo, T., Wei, W., Ruan, R., Hu, B., 2011. Mixotrophic cultivation of Chlorella vulgaris and its potential application for the oil accumulation from non-sugar materials. Biomass Bioenergy 35, 2245–2253. doi:10.1016/j.biombioe.2011.02.036

Hicks, N., Vik, U., Taylor, P., Ladoukakis, E., Park, J., Kolisis, F., Jakobsen, K.S., 2017. Using Prokaryotes for Carbon Capture Storage. Trends Biotechnol. 35, 22–32. doi:10.1016/j.tibtech.2016.06.011

Ho, D.P., Ngo, H.H., Guo, W., 2014. A mini review on renewable sources for biofuel. Bioresour. Technol. 169, 742–749. doi:10.1016/j.biortech.2014.07.022

Hoffmann, M., Marxen, K., Schulz, R., Vanselow, K.H., 2010. TFA and EPA productivities of Nannochloropsis salina influenced by temperature and nitrate stimuli in turbidostatic controlled experiments. Mar. Drugs 8, 2526–2545. doi:10.3390/md8092526

Hu, Q., Kurano, N., Kawachi, M., Iwasaki, I., Miyachi, S., 1998. Ultrahigh-cell-density culture of a marine green alga Chlorococcum littorale in a flat-plate photobioreactor. Appl. Microbiol. Biotechnol. 49, 655–662. doi:10.1007/s002530051228

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. Cell Mol. Biol. 54, 621–639. doi:10.1111/j.1365-313X.2008.03492.x

Hu, Y., Liu, X., Zhou, Z., Liu, W., Xu, M., 2017. Pelletization of MgO-based sorbents for intermediate temperature CO_2 capture. Fuel 187, 328–337. doi:10.1016/j.fuel.2016.09.066

Iverson, T.M., 2006. Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis. Curr. Opin. Chem. Biol. 10, 91–100. doi:10.1016/j.cbpa.2006.02.013

Jacob-Lopes, E., Scoparo, C.H.G., Lacerda, L.M.C.F., Franco, T.T., 2009. Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors. Chem. Eng. Process. Process Intensif. 48, 306–310. doi:10.1016/j.cep.2008.04.007

Janssen, M., Bresser, L. de, Baijens, T., Tramper, J., Mur, L.R., Snel, J.F.H., Wijffels, R.H., 2000. Scale-up aspects of photobioreactors: effects of mixing-induced light/dark cycles. J. Appl. Phycol. 12, 225–237. doi:10.1023/A:1008151526680

Jazzar, S., Olivares-Carrillo, P., Pérez de los Ríos, A., Marzouki, M.N., Acién-Fernández, F.G., Fernández-Sevilla, J.M., Molina-Grima, E., Smaali, I., Quesada-Medina, J., 2015. Direct supercritical methanolysis of wet and dry unwashed marine microalgae (Nannochloropsis gaditana) to biodiesel. Appl. Energy 148, 210–219. doi:10.1016/j.apenergy.2015.03.069

Jiménez, C., Cossı́o, B.R., Niell, F.X., 2003. Relationship between physicochemical variables and productivity in open ponds for the production of Spirulina: a predictive model of algal yield. Aquaculture 221, 331–345. doi:10.1016/S0044-8486(03)00123-6

Kan, G., Shi, C., Wang, X., Xie, Q., Wang, M., Wang, X., Miao, J., 2012. Acclimatory responses to high-salt stress in Chlamydomonas (Chlorophyta, Chlorophyceae) from Antarctica. Acta Oceanol. Sin. 31, 116–124. doi:10.1007/s13131-012-0183-2

Kang, N.K., Lee, B., Choi, G.-G., Moon, M., Park, M.S., Lim, J., Yang, J.-W., 2014. Enhancing lipid productivity of Chlorella vulgaris using oxidative stress by TiO2 nanoparticles. Korean J. Chem. Eng. 31, 861–867. doi:10.1007/s11814-013-0258-6

Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., Kumar, P., 2016. Microalgae: An emerging source of energy based bio-products and a solution for environmental issues. Renew. Sustain. Energy Rev. doi:10.1016/j.rser.2016.10.028

Kemper, J., 2015. Biomass and carbon dioxide capture and storage: A review. Int. J. Greenh. Gas Control 40, 401–430. doi:10.1016/j.ijggc.2015.06.012

Ketheesan, B., Nirmalakhandan, N., 2012. Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor. Bioresour. Technol. 108, 196–202. doi:10.1016/j.biortech.2011.12.146

Ketheesan, B., Nirmalakhandan, N., 2011. Development of a new airlift-driven raceway reactor for algal cultivation. Appl. Energy, Special Issue of Energy from algae: Current status and future trends 88, 3370–3376. doi:10.1016/j.apenergy.2010.12.034

Khoo, C.G., Lam, M.K., Lee, K.T., 2016. Pilot-scale semi-continuous cultivation of microalgae Chlorella vulgaris in bubble column photobioreactor (BC-PBR): Hydrodynamics and gas–liquid mass transfer study. Algal Res. 15, 65–76. doi:10.1016/j.algal.2016.02.001

Khozin-Goldberg, I., Cohen, Z., 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte Monodus subterraneus. Phytochemistry 67, 696–701. doi:10.1016/j.phytochem.2006.01.010

Kim, C.W., Moon, M., Park, W.-K., Yoo, G., Choi, Y.-E., Yang, J.-W., 2014. Energy-efficient cultivation of Chlamydomonas reinhardtii for lipid accumulation under flashing illumination conditions. Biotechnol. Bioprocess Eng. 19, 150–158. doi:10.1007/s12257-013-0468-0

Kim, H., Kim, Y.H., Kang, S.-G., Park, Y.-G., 2016. Development of environmental impact monitoring protocol for offshore carbon capture and storage (CCS): A biological perspective. Environ. Impact Assess. Rev. 57, 139–150. doi:10.1016/j.eiar.2015.11.004

Kirrolia, A., Bishnoi, N.R., Singh, R., 2014. Response surface methodology as a decision-making tool for optimization of culture conditions of green microalgae Chlorella spp. for biodiesel production. Ann. Microbiol. 64, 1133–1147. doi:10.1007/s13213-013-0752-4

Klinthong, W., 2015. A Review: Microalgae and Their Applications in CO_2 Capture and Renewable Energy. Aerosol Air Qual. Res. doi:10.4209/aaqr.2014.11.0299

Knežević, M., Povrenović, D., 2015. Influence of fluid-mechanical parameters on volumetric mass transfer coefficient in a spout–fluid bed with a draft tube. Chem. Eng. Sci. 134, 129–137. doi:10.1016/j.ces.2015.04.030

Kroth, P.G., 2015. The biodiversity of carbon assimilation. J. Plant Physiol., Plant Physiology meets Biodiversity 172, 76–81. doi:10.1016/j.jplph.2014.07.021

Kroumov, A.D., Módenes, A.N., Trigueros, D.E.G., Espinoza-Quiñones, F.R., Borba, C.E., Scheufele, F.B., Hinterholz, C.L., 2016. A systems approach for CO_2 fixation from flue gas by microalgae—Theory review. Process Biochem. 51, 1817–1832. doi:10.1016/j.procbio.2016.05.019

Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., van Langenhove, H., 2010. Enhanced CO(2) fixation and biofuel production via microalgae: recent developments and future directions. Trends Biotechnol. 28, 371–380. doi:10.1016/j.tibtech.2010.04.004

Kumar, K., Das, D., 2012. Growth characteristics of Chlorella sorokiniana in airlift and bubble column photobioreactors. Bioresour. Technol. 116, 307–313. doi:10.1016/j.biortech.2012.03.074

Kumar, K., Dasgupta, C.N., Nayak, B., Lindblad, P., Das, D., 2011. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour. Technol. 102, 4945–4953. doi:10.1016/j.biortech.2011.01.054

Kumar, K., Ghosh, S., Angelidaki, I., Holdt, S.L., Karakashev, D.B., Morales, M.A., Das, D., 2016. Recent developments on biofuels production from microalgae and macroalgae. Renew. Sustain. Energy Rev. 65, 235–249. doi:10.1016/j.rser.2016.06.055

Kumar, K., Mishra, S.K., Shrivastav, A., Park, M.S., Yang, J.-W., 2015. Recent trends in the mass cultivation of algae in raceway ponds. Renew. Sustain. Energy Rev. 51, 875–885. doi:10.1016/j.rser.2015.06.033

Laamanen, C.A., Senhorinho, G.N.A., Ross, G.M., Scott, J.A., 2016. Heat-aided flocculation for flotation harvesting of microalgae. Algal Res. 20, 213–217. doi:10.1016/j.algal.2016.10.019

Laamanen, C.A., Shang, H., Ross, G.M., Scott, J.A., 2014. A model for utilizing industrial off-gas to support microalgae cultivation for biodiesel in cold climates. Energy Convers. Manag. 88, 476–483. doi:10.1016/j.enconman.2014.08.047

Lam, M.K., Lee, K.T., Mohamed, A.R., 2012. Current status and challenges on microalgae-based carbon capture. Int. J. Greenh. Gas Control 10, 456–469. doi:10.1016/j.ijggc.2012.07.010

Lara-Gil, J.A., Senés-Guerrero, C., Pacheco, A., 2016. Cement flue gas as a potential source of nutrients during CO_2 mitigation by microalgae. Algal Res. 17, 285–292. doi:10.1016/j.algal.2016.05.017

Laumb, J.D., Kay, J.P., Holmes, M.J., Cowan, R.M., Azenkeng, A., Heebink, L.V., Hanson, S.K., Jensen, M.D., Letvin, P.A., Raymond, L.J., 2013. Economic and Market

Analysis of CO_2 Utilization Technologies – Focus on CO_2 derived from North Dakota lignite. Energy Procedia 37, 6987–6998. doi:10.1016/j.egypro.2013.06.632

Lebeau, T., Robert, J.-M., 2003. Diatom cultivation and biotechnologically relevant products. Part I: Cultivation at various scales. Appl. Microbiol. Biotechnol. 60, 612–623. doi:10.1007/s00253-002-1176-4

Lee, K.T., Bhatia, S., Mohamed, A.R., 2005. Preparation and characterization of sorbents prepared from ash (waste material) for sulfur dioxide (SO2) removal. J. Mater. Cycles Waste Manag. 7, 16–23. doi:10.1007/s10163-004-0121-2

Lee, S.-Y., Lee, J.-U., Lee, I.-B., Han, J., 2017. Design under uncertainty of carbon capture and storage infrastructure considering cost, environmental impact, and preference on risk. Appl. Energy 189, 725–738. doi:10.1016/j.apenergy.2016.12.066

Li, S., Ding, J., Zhang, X., Cheng, D., Hu, X., Li, X., 2016. A feasible energy-saving analysis of a new system for CO_2 cryogenic capture. Int. J. Low-Carbon Technol. 11, 235–239. doi:10.1093/ijlct/ctt065

Li, S., Luo, S., Guo, R., 2013. Efficiency of CO_2 fixation by microalgae in a closed raceway pond. Bioresour. Technol. 136, 267–272. doi:10.1016/j.biortech.2013.03.025

Li, Y., Han, D., Hu, G., Sommerfeld, M., Hu, Q., 2010. Inhibition of starch synthesis results in overproduction of lipids in Chlamydomonas reinhardtii. Biotechnol. Bioeng. 107, 258–268. doi:10.1002/bit.22807

Liu, G., Qiao, L., Zhang, H., Zhao, D., Su, X., 2014. The effects of illumination factors on the growth and HCO3− fixation of microalgae in an experiment culture system. Energy 78, 40–47. doi:10.1016/j.energy.2014.05.043

Liu, L., Qiu, W., Sanders, E.S., Ma, C., Koros, W.J., 2016. Post-combustion carbon dioxide capture via 6FDA/BPDA-DAM hollow fiber membranes at sub-ambient temperatures. J. Membr. Sci. 510, 447–454. doi:10.1016/j.memsci.2016.03.027

Liu, W., Huang, Z., Li, P., Xia, J., Chen, B., 2012. Formation of triacylglycerol in Nitzschia closterium f. minutissima under nitrogen limitation and possible physiological and biochemical mechanisms. J. Exp. Mar. Biol. Ecol. 418–419, 24–29. doi:10.1016/j.jembe.2012.03.005

Liu, Z.-Y., Wang, G.-C., Zhou, B.-C., 2008. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour. Technol., Exploring Horizons in Biotechnology: A Global Venture 99, 4717–4722. doi:10.1016/j.biortech.2007.09.073

Lombardi, A.T., Wangersky, P.J., 1995. Particulate lipid class composition of three marine phytoplankters Chaetoceros gracilis, Isochrysis galbana (Tahiti) and Dunaliella tertiolecta grown in batch culture. Hydrobiologia 306, 1–6. doi:10.1007/BF00007853

Lowrey, J., Brooks, M.S., McGinn, P.J., 2015. Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges—a critical review. J. Appl. Phycol. 27, 1485–1498. doi:10.1007/s10811-014-0459-3

Luo, H.-P., Al-Dahhan, M.H., 2012. Airlift column photobioreactors for Porphyridium sp. culturing: part I. effects of hydrodynamics and reactor geometry. Biotechnol. Bioeng. 109, 932–941. doi:10.1002/bit.24361

Maberly, S.C., Ball, L.A., Raven, J.A., Sültemeyer, D., 2009. Inorganic Carbon Acquisition by Chrysophytes1. J. Phycol. 45, 1052–1061. doi:10.1111/j.1529-8817.2009.00734.x

Machado, M.D., Soares, E.V., 2016. Short- and Long-Term Exposure to Heavy Metals Induced Oxidative Stress Response in Pseudokirchneriella subcapitata. CLEAN – Soil Air Water 44, 1578–1583. doi:10.1002/clen.201600020

Madden, D.G., Scott, H.S., Kumar, A., Chen, K.-J., Sanii, R., Bajpai, A., Lusi, M., Curtin, T., Perry, J.J., Zaworotko, M.J., 2017. Flue-gas and direct-air capture of CO_2 by porous metal–organic materials. Phil Trans R Soc A 375, 20160025. doi:10.1098/rsta.2016.0025

Mandal, S., Mallick, N., 2009. Microalga Scenedesmus obliquus as a potential source for biodiesel production. Appl. Microbiol. Biotechnol. 84, 281–291. doi:10.1007/s00253-009-1935-6

Mandal, S., Sarkar, B., Bolan, N., Novak, J., Ok, Y.S., Zwieten, L.V., Singh, B.P., Kirkham, M.B., Choppala, G., Spokas, K., Naidu, R., 2016. Designing advanced biochar products for maximizing greenhouse gas mitigation potential. Crit. Rev. Environ. Sci. Technol. 46, 1367–1401. doi:10.1080/10643389.2016.1239975

Manzello, D.P., Eakin, C.M., Glynn, P.W., 2017. Effects of Global Warming and Ocean Acidification on Carbonate Budgets of Eastern Pacific Coral Reefs, in: Glynn, P.W., Manzello, D.P., Enochs, I.C. (Eds.), Coral Reefs of the Eastern Tropical Pacific, Coral Reefs of the World. Springer Netherlands, pp. 517–533. doi:10.1007/978-94-017-7499-4_18

Maqsood, K., Ali, A., Shariff, A.B.M., Ganguly, S., 2017. Process intensification using mixed sequential and integrated hybrid cryogenic distillation network for purification of high CO_2 natural gas. Chem. Eng. Res. Des. 117, 414–438. doi:10.1016/j.cherd.2016.10.011

Markou, G., Nerantzis, E., 2013. Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnol. Adv. 31, 1532–1542. doi:10.1016/j.biotechadv.2013.07.011

Maroneze, M.M., Siqueira, S.F., Vendruscolo, R.G., Wagner, R., de Menezes, C.R., Zepka, L.Q., Jacob-Lopes, E., 2016. The role of photoperiods on photobioreactors – A

potential strategy to reduce costs. Bioresour. Technol. 219, 493–499. doi:10.1016/j.biortech.2016.08.003

Marques, L.C. do C., Pimentel, D.M., 2016. Pitfalls of CO_2 Injection in Enhanced Oil Recovery. Appl. Mech. Mater. 830, 125–133. doi:10.4028/www.scientific.net/AMM.830.125

Marshall, J.P., 2016. Disordering fantasies of coal and technology: Carbon capture and storage in Australia. Energy Policy 99, 288–298. doi:10.1016/j.enpol.2016.05.044

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: A review. Renew. Sustain. Energy Rev. 14, 217–232. doi:10.1016/j.rser.2009.07.020

Matsumoto, H., Hamasaki, A., Sioji, N., Ikuta, Y., 1997. Influence of CO_2, SO2 and NO in Flue Gas on Microalgae Productivity. J. Chem. Eng. Jpn. 30, 620–624. doi:10.1252/jcej.30.620

Mayer, A.M., Zuri, U., Shain, Y., Ginzburg, H., 1964. Problems of design and ecological considerations in mass culture of algae. Biotechnol. Bioeng. 6, 173–190. doi:10.1002/bit.260060207

Mehrpooya, M., Esfilar, R., Moosavian, S.M.A., 2017. Introducing a novel air separation process based on cold energy recovery of LNG integrated with coal gasification, transcritical carbon dioxide power cycle and cryogenic CO_2 capture. J. Clean. Prod. 142, Part 4, 1749–1764. doi:10.1016/j.jclepro.2016.11.112

Meijer, H.J.G., van Himbergen, J.A.J., Musgrave, A., Munnik, T., 2016. Acclimation to salt modifies the activation of several osmotic stress-activated lipid signalling pathways in Chlamydomonas. Phytochemistry. doi:10.1016/j.phytochem.2016.12.014

Mendoza, J.L., Granados, M.R., de Godos, I., Acién, F.G., Molina, E., Heaven, S., Banks, C.J., 2013. Oxygen transfer and evolution in microalgal culture in open raceways. Bioresour. Technol. 137, 188–195. doi:10.1016/j.biortech.2013.03.127

Merriman, L., Moix, A., Beitle, R., Hestekin, J., 2014. Carbon dioxide gas delivery to thin-film aqueous systems via hollow fiber membranes. Chem. Eng. J. 253, 165–173. doi:10.1016/j.cej.2014.04.075

Meyer, M., Griffiths, H., 2013. Origins and diversity of eukaryotic CO_2 -concentrating mechanisms: lessons for the future. J. Exp. Bot. 64, 769–786. doi:10.1093/jxb/ers390

Meyer, M.T., McCormick, A.J., Griffiths, H., 2016. Will an algal CO_2 -concentrating mechanism work in higher plants? Curr. Opin. Plant Biol., SI: 31: Physiology and metabolism 2016 31, 181–188. doi:10.1016/j.pbi.2016.04.009

Michels, M.H.A., Goot, A.J. van der, Vermuë, M.H., Wijffels, R.H., 2016. Cultivation of shear stress sensitive and tolerant microalgal species in a tubular photobioreactor

equipped with a centrifugal pump. J. Appl. Phycol. 28, 53–62. doi:10.1007/s10811-015-0559-8

Mirón, A.S., García, M.C.C., Gómez, A.C., Camacho, F.G., Grima, E.M., Chisti, Y., 2003. Shear stress tolerance and biochemical characterization of Phaeodactylum tricornutum in quasi steady-state continuous culture in outdoor photobioreactors. Biochem. Eng. J. 16, 287–297. doi:10.1016/S1369-703X(03)00072-X

Mitra, M., Patidar, S.K., Mishra, S., 2015. Integrated process of two stage cultivation of Nannochloropsis sp. for nutraceutically valuable eicosapentaenoic acid along with biodiesel. Bioresour. Technol. 193, 363–369. doi:10.1016/j.biortech.2015.06.033

Molina, E., Contreras, A., Chisti, Y., 1999. Gas Holdup, Liquid Circulation and Mixing Behaviour of Viscous Newtonian Media in a Split-Cylinder Airlift Bioreactor. Food Bioprod. Process. 77, 27–32. doi:10.1205/096030899532222

Mondal, M., Khanra, S., Tiwari, O. n., Gayen, K., Halder, G. n., 2016. Role of carbonic anhydrase on the way to biological carbon capture through microalgae—A mini review. Environ. Prog. Sustain. Energy 35, 1605–1615. doi:10.1002/ep.12394

Morais, M.G. de, Costa, J.A.V., 2007. Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol. Lett. 29, 1349–1352. doi:10.1007/s10529-007-9394-6

Moreira, D., Pires, J.C.M., 2016. Atmospheric CO_2 capture by algae: Negative carbon dioxide emission path. Bioresour. Technol., Waste Biorefinery - Advocating Circular Economy 215, 371–379. doi:10.1016/j.biortech.2016.03.060

Moroney, J.V., Ynalvez, R.A., 2007. Proposed carbon dioxide concentrating mechanism in Chlamydomonas reinhardtii. Eukaryot. Cell 6, 1251–1259. doi:10.1128/EC.00064-07

Munson, B.R., Young, D.F., Okiishi, T.H., 1995. Fundamentals of fluid mechanics. Oceanogr. Lit. Rev. 10, 831.

Napan, K., Teng, L., Quinn, J.C., Wood, B.D., 2015. Impact of heavy metals from flue gas integration with microalgae production. Algal Res. 8, 83–88. doi:10.1016/j.algal.2015.01.003

Napolitano, G.E., 1994. The relationship of lipids with light and xhlorophyll measurements in freshwater algae and periphyton1. J. Phycol. 30, 943–950. doi:10.1111/j.0022-3646.1994.00943.x

Narala, R.R., Garg, S., Sharma, K.K., Thomas-Hall, S.R., Deme, M., Li, Y., Schenk, P.M., 2016. Comparison of Microalgae Cultivation in Photobioreactor, Open Raceway Pond, and a Two-Stage Hybrid System. Front. Energy Res. 4. doi:10.3389/fenrg.2016.00029

Nikolaidis, G.N., Kikkinides, E.S., Georgiadis, M.C., 2017. An Integrated Two-stage P/VSA Process for Post-Combustion CO_2 Capture Using Combinations of Adsorbents Zeolite 13X and Mg-MOF-74. Ind. Eng. Chem. Res. doi:10.1021/acs.iecr.6b04270

Novek, E.J., Shaulsky, E., Fishman, Z.S., Pfefferle, L.D., Elimelech, M., 2016. Low-Temperature Carbon Capture Using Aqueous Ammonia and Organic Solvents. Environ. Sci. Technol. Lett. 3, 291–296. doi:10.1021/acs.estlett.6b00253

Olofsson, M., Lamela, T., Nilsson, E., Bergé, J.P., del Pino, V., Uronen, P., Legrand, C., 2012. Seasonal Variation of Lipids and Fatty Acids of the Microalgae Nannochloropsis oculata Grown in Outdoor Large-Scale Photobioreactors. Energies 5, 1577–1592. doi:10.3390/en5051577

Olofsson, M., Lindehoff, E., Frick, B., Svensson, F., Legrand, C., 2015. Baltic Sea microalgae transform cement flue gas into valuable biomass. Algal Res. 11, 227–233. doi:10.1016/j.algal.2015.07.001

Oncel, S., Sukan, F.V., 2008. Comparison of two different pneumatically mixed column photobioreactors for the cultivation of Artrospira platensis (Spirulina platensis). Bioresour. Technol., Exploring Horizons in Biotechnology: A Global Venture 99, 4755–4760. doi:10.1016/j.biortech.2007.09.068

Ono, E., Cuello, J.L., 2004. Design parameters of solar concentrating systems for CO_2 -mitigating algal photobioreactors. Energy, 6th International Conference on Greenhouse Gas Control Technologies 29, 1651–1657. doi:10.1016/j.energy.2004.03.067

Orefice, I., Chandrasekaran, R., Smerilli, A., Corato, F., Caruso, T., Casillo, A., Corsaro, M.M., Piaz, F.D., Ruban, A.V., Brunet, C., 2016. Light-induced changes in the photosynthetic physiology and biochemistry in the diatom Skeletonema marinoi. Algal Res. Complete, 1–13. doi:10.1016/j.algal.2016.04.013

Osundeko, O., Davies, H., Pittman, J.K., 2013. Oxidative stress-tolerant microalgae strains are highly efficient for biofuel feedstock production on wastewater. Biomass Bioenergy 56, 284–294. doi:10.1016/j.biombioe.2013.05.027

Osundeko, O., Dean, A.P., Davies, H., Pittman, J.K., 2014. Acclimation of Microalgae to Wastewater Environments Involves Increased Oxidative Stress Tolerance Activity. Plant Cell Physiol. 55, 1848–1857. doi:10.1093/pcp/pcu113

Pancha, I., Chokshi, K., Maurya, R., Trivedi, K., Patidar, S.K., Ghosh, A., Mishra, S., 2015. Salinity induced oxidative stress enhanced biofuel production potential of microalgae Scenedesmus sp. CCNM 1077. Bioresour. Technol. 189, 341–348. doi:10.1016/j.biortech.2015.04.017

Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., Simões, M., 2012. Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept. Renew. Sustain. Energy Rev. 16, 3043–3053. doi:10.1016/j.rser.2012.02.055

Plackett, R.L., Burman, J.P., 1946. The Design of Optimum Multifactorial Experiments. Biometrika. 33, 305. doi:10.2307/2332195.

Posada, J.A., Brentner, L.B., Ramirez, A., Patel, M.K., 2016. Conceptual design of sustainable integrated microalgae biorefineries: Parametric analysis of energy use, greenhouse gas emissions and techno-economics. Algal Res. 17, 113–131. doi:10.1016/j.algal.2016.04.022

Přibyl, P., Pilný, J., Cepák, V., Kaštánek, P., 2016. The role of light and nitrogen in growth and carotenoid accumulation in Scenedesmus sp. Algal Res. 16, 69–75. doi:10.1016/j.algal.2016.02.028

Prussi, M., Buffi, M., Casini, D., Chiaramonti, D., Martelli, F., Carnevale, M., Tredici, M.R., Rodolfi, L., 2014. Experimental and numerical investigations of mixing in raceway ponds for algae cultivation. Biomass Bioenergy 67, 390–400. doi:10.1016/j.biombioe.2014.05.024

Pruvost, J., Van Vooren, G., Le Gouic, B., Couzinet-Mossion, A., Legrand, J., 2011. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. Bioresour. Technol., Special Issue: Biofuels - II: Algal Biofuels and Microbial Fuel Cells 102, 150–158. doi:10.1016/j.biortech.2010.06.153

Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635–648. doi:10.1007/s00253-004-1647-x

Putt, R., Singh, M., Chinnasamy, S., Das, K.C., 2011. An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. Bioresour. Technol. 102, 3240–3245. doi:10.1016/j.biortech.2010.11.029

Ra, C.-H., Kang, C.-H., Jung, J.-H., Jeong, G.-T., Kim, S.-K., 2016. Effects of light-emitting diodes (LEDs) on the accumulation of lipid content using a two-phase culture process with three microalgae. Bioresour. Technol. 212, 254–261. doi:10.1016/j.biortech.2016.04.059

Ramanan, R., Kannan, K., Deshkar, A., Yadav, R., Chakrabarti, T., 2010. Enhanced algal CO_2 sequestration through calcite deposition by Chlorella sp. and Spirulina platensis in a mini-raceway pond. Bioresour. Technol. 101, 2616–2622. doi:10.1016/j.biortech.2009.10.061

Ranathunga, A.S., Perera, M.S.A., Ranjith, P.G., Wei, C.H., 2017. An experimental investigation of applicability of CO_2 enhanced coal bed methane recovery to low rank coal. Fuel 189, 391–399. doi:10.1016/j.fuel.2016.10.116

Rao, A.R., Dayananda, C., Sarada, R., Shamala, T.R., Ravishankar, G.A., 2007. Effect of salinity on growth of green alga Botryococcus braunii and its constituents. Bioresour. Technol. 98, 560–564. doi:10.1016/j.biortech.2006.02.007

Raven, J.A., Beardall, J., 2016. The ins and outs of CO_2. J. Exp. Bot. 67, 1–13. doi:10.1093/jxb/erv451

Raven, J.A., Giordano, M., Beardall, J., Maberly, S.C., 2012. Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Phil Trans R Soc B 367, 493–507. doi:10.1098/rstb.2011.0212

Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Appl. Energy 103, 444–467. doi:10.1016/j.apenergy.2012.10.004

Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., de Lasa, H., 2013. Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—A review. Renew. Sustain. Energy Rev. 27, 622–653. doi:10.1016/j.rser.2013.05.063

Rengel, A., Zoughaib, A., Dron, D., Clodic, D., 2012. Hydrodynamic study of an internal airlift reactor for microalgae culture. Appl. Microbiol. Biotechnol. 93, 117–129. doi:10.1007/s00253-011-3398-9

Řezanka, T., Nedbalová, L., Lukavský, J., Střížek, A., Sigler, K., 2017. Pilot cultivation of the green alga Monoraphidium sp. producing a high content of polyunsaturated fatty acids in a low-temperature environment. Algal Res. 22, 160–165. doi:10.1016/j.algal.2016.12.017

Richardson, J.W., Johnson, M.D., Outlaw, J.L., 2012. Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the Southwest. Algal Res. 1, 93–100. doi:10.1016/j.algal.2012.04.001

Rocchetta, I., Mazzuca, M., Conforti, V., Ruiz, L., Balzaretti, V., de Molina, M. del C.R., 2006. Effect of chromium on the fatty acid composition of two strains of Euglena gracilis. Environ. Pollut. 141, 353–358. doi:10.1016/j.envpol.2005.08.035

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102, 100–112. doi:10.1002/bit.22033

Roleda, M.Y., Slocombe, S.P., Leakey, R.J.G., Day, J.G., Bell, E.M., Stanley, M.S., 2013. Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresour. Technol. 129, 439–449. doi:10.1016/j.biortech.2012.11.043

Ruangsomboon, S., 2012. Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, Botryococcus braunii KMITL 2. Bioresour. Technol., Special Issue: Innovative Researches on Algal Biomass 109, 261–265. doi:10.1016/j.biortech.2011.07.025

Ruangsomboon, S., Ganmanee, M., Choochote, S., 2013. Effects of different nitrogen, phosphorus, and iron concentrations and salinity on lipid production in newly isolated strain of the tropical green microalga, Scenedesmus dimorphus KMITL. J. Appl. Phycol. 25, 867–874. doi:10.1007/s10811-012-9956-4

Rubin, E.S., Mantripragada, H., Marks, A., Versteeg, P., Kitchin, J., 2012. The outlook for improved carbon capture technology. Prog. Energy Combust. Sci. 38, 630–671. doi:10.1016/j.pecs.2012.03.003

Ryan, T.P., 2007. Modern experimental design. Hoboken, N.J: Wiley-Interscience.

Ryu, H.J., Oh, K.K., Kim, Y.S., 2009. Optimization of the influential factors for the improvement of CO_2 utilization efficiency and CO_2 mass transfer rate. J. Ind. Eng. Chem. 15, 471–475. doi:10.1016/j.jiec.2008.12.012

Sage, R.F., Stata, M., 2015. Photosynthetic diversity meets biodiversity: The C4 plant example. J. Plant Physiol., Plant Physiology meets Biodiversity 172, 104–119. doi:10.1016/j.jplph.2014.07.024

Sánchez Mirón, A., Cerón García, M.-C., García Camacho, F., Molina Grima, E., Chisti, Y., 2004. Mixing in Bubble Column and Airlift Reactors. Chem. Eng. Res. Des. 82, 1367–1374. doi:10.1205/cerd.82.10.1367.46742

Sánchez Mirón, A., García Camacho, F., Contreras Gómez, A., Grima, E.M., Chisti, Y., 2000. Bubble-column and airlift photobioreactors for algal culture. AIChE J. 46, 1872–1887. doi:10.1002/aic.690460915

Santos, A.M., Wijffels, R.H., Lamers, P.P., 2014. pH-upshock yields more lipids in nitrogen-starved Neochloris oleoabundans. Bioresour. Technol. 152, 299–306. doi:10.1016/j.biortech.2013.10.079

Sayegh, F.A.Q., Montagnes, D.J.S., 2011. Temperature shifts induce intraspecific variation in microalgal production and biochemical composition. Bioresour. Technol. 102, 3007–3013. doi:10.1016/j.biortech.2010.10.011

Seyed Hosseini, N., Shang, H., Ross, G.M., Scott, J.A., 2016. Comparative analysis of top-lit bubble column and gas-lift bioreactors for microalgae-sourced biodiesel production. Energy Convers. Manag. 130, 230–239. doi:10.1016/j.enconman.2016.10.048

Seyed Hosseini, N., Shang, H., Ross, G.M., Scott, J.A., 2015. Microalgae cultivation in a novel top-lit gas-lift open bioreactor. Bioresour. Technol. 192, 432–440. doi:10.1016/j.biortech.2015.05.092

Sforza, E., Simionato, D., Giacometti, G.M., Bertucco, A., Morosinotto, T., 2012. Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors. PloS One 7, e38975. doi:10.1371/journal.pone.0038975

Shang, H., Scott, J.A., 2011. Minimizing leakage from a smelter off-gas system by enhanced control. J. Clean. Prod. 19, 70–77. doi:10.1016/j.jclepro.2010.08.015

Shang, H., Scott, J.A., Shepherd, S.H., Ross, G.M., 2010. A dynamic thermal model for heating microalgae incubator ponds using off-gas. Chem. Eng. Sci. 65, 4591–4597. doi:10.1016/j.ces.2010.04.042

Shelton, J.L., McIntosh, J.C., Hunt, A.G., Beebe, T.L., Parker, A.D., Warwick, P.D., Drake II, R.M., McCray, J.E., 2016. Determining CO_2 storage potential during miscible CO_2 enhanced oil recovery: Noble gas and stable isotope tracers. Int. J. Greenh. Gas Control 51, 239–253. doi:10.1016/j.ijggc.2016.05.008

Shen, Y., Yuan, W., Pei, Z.J., Wu, Q., Mao, E., 2009. Microalgae mass production methods. Trans. ASABE 52, 1275–1287.

Sibi, G., Anuraag, T.S., Bafila, G., 2014. Copper stress on cellular contents and fatty acid profiles in Chlorella species. OnLine J. Biol. Sci. 14, 209–217. doi:10.3844/ojbsci.2014.209.217

Sibi, G., Shetty, V., Mokashi, K., 2016. Enhanced lipid productivity approaches in microalgae as an alternate for fossil fuels – A review. J. Energy Inst. 89, 330–334. doi:10.1016/j.joei.2015.03.008

Singh, P., Kumari, S., Guldhe, A., Misra, R., Rawat, I., Bux, F., 2016. Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew. Sustain. Energy Rev. 55, 1–16. doi:10.1016/j.rser.2015.11.001

Singh, R.N., Sharma, S., 2012. Development of suitable photobioreactor for algae production – A review. Renew. Sustain. Energy Rev. 16, 2347–2353. doi:10.1016/j.rser.2012.01.026

Singh, S.K., Sundaram, S., Sinha, S., Rahman, M.A., Kapur, S., 2016. Recent advances in CO_2 uptake and fixation mechanism of cyanobacteria and microalgae. Crit. Rev. Environ. Sci. Technol. 46, 1297–1323. doi:10.1080/10643389.2016.1217911

Singh, V., Tiwari, A., Das, M., 2016. Phyco-remediation of industrial waste-water and flue gases with algal-diesel engenderment from micro-algae: A review. Fuel 173, 90–97. doi:10.1016/j.fuel.2016.01.031

Skorupskaite, V., Makareviciene, V., Levisauskas, D., 2015. Optimization of mixotrophic cultivation of microalgae Chlorella sp. for biofuel production using response surface methodology. Algal Res. 7, 45–50. doi:10.1016/j.algal.2014.12.001

Slegers, P.M., Lösing, M.B., Wijffels, R.H., van Straten, G., van Boxtel, A.J.B., 2013. Scenario evaluation of open pond microalgae production. Algal Res. 2, 358–368. doi:10.1016/j.algal.2013.05.001

Solimeno, A., Samsó, R., Uggetti, E., Sialve, B., Steyer, J.-P., Gabarró, A., García, J., 2015. New mechanistic model to simulate microalgae growth. Algal Res. 12, 350–358. doi:10.1016/j.algal.2015.09.008

Sondak, C.F.A., Ang, P.O., Beardall, J., Bellgrove, A., Boo, S.M., Gerung, G.S., Hepburn, C.D., Hong, D.D., Hu, Z., Kawai, H., Largo, D., Lee, J.A., Lim, P.-E., Mayakun, J., Nelson, W.A., Oak, J.H., Phang, S.-M., Sahoo, D., Peerapornpis, Y., Yang, Y., Chung, I.K., 2016. Carbon dioxide mitigation potential of seaweed aquaculture beds (SABs). J. Appl. Phycol. 1–11. doi:10.1007/s10811-016-1022-1

Song, C., Liu, Q., Ji, N., Deng, S., Zhao, J., Kitamura, Y., 2017. Advanced cryogenic CO_2 capture process based on Stirling coolers by heat integration. Appl. Therm. Eng. 114, 887–895. doi:10.1016/j.applthermaleng.2016.12.049

Song, M., Pei, H., Hu, W., Han, F., Ji, Y., Ma, G., Han, L., 2014. Growth and lipid accumulation properties of microalgal Phaeodactylum tricornutum under different gas liquid ratios. Bioresour. Technol., Special Issue: CESE 2013 & Special Issue: ICABB 2013 165, 31–37. doi:10.1016/j.biortech.2014.03.070

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96. doi:10.1263/jbb.101.87

Sreedhar, I., Vaidhiswaran, R., Kamani, B.M., Venugopal, A., 2017. Process and engineering trends in membrane based carbon capture. Renew. Sustain. Energy Rev. 68, Part 1, 659–684. doi:10.1016/j.rser.2016.10.025

Su, Z., Kang, R., Shi, S., Cong, W., Cai, Z., 2008. An economical device for carbon supplement in large-scale micro-algae production. Bioprocess Biosyst. Eng. 31, 641–645. doi:10.1007/s00449-008-0214-8

Sutherland, D.L., Turnbull, M.H., Craggs, R.J., 2014. Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. Water Res. 53, 271–281. doi:10.1016/j.watres.2014.01.025

Szalaj, D., Orte, M.R.D., Goulding, T.A., Medeiros, I.D., DelValls, T.A., Cesar, A., 2017. The effects of ocean acidification and a carbon dioxide capture and storage leak on the early life stages of the marine mussel Perna perna (Linneaus, 1758) and metal bioavailability. Environ. Sci. Pollut. Res. 24, 765–781. doi:10.1007/s11356-016-7863-y

Takagi, M., Karseno, null, Yoshida, T., 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. J. Biosci. Bioeng. 101, 223–226. doi:10.1263/jbb.101.223

Takagi, M., Watanabe, K., Yamaberi, K., Yoshida, T., 2000. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of Nannochloris sp. UTEX LB1999. Appl. Microbiol. Biotechnol. 54, 112–117.

Talaia, M.A.R., 2007. Terminal Velocity of a bubble rise in a liquid. Int J Math Comput Phys Electr Comput Eng 1, 220–224.

Tanaka, S., Ikeda, K., Miyasaka, H., Shioi, Y., Suzuki, Y., Tamoi, M., Takeda, T., Shigeoka, S., Harada, K., Hirata, K., 2011. Comparison of three Chlamydomonas strains which show distinctive oxidative stress tolerance. J. Biosci. Bioeng. 112, 462–468. doi:10.1016/j.jbiosc.2011.07.019

Tang, D., Han, W., Li, P., Miao, X., Zhong, J., 2011. CO_2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO_2 levels. Bioresour. Technol. 102, 3071–3076. doi:10.1016/j.biortech.2010.10.047

Tatsuzawa, H., Takizawa, E., Wada, M., Yamamoto, Y., 1996. Fatty Acid and Lipid Composition of the Acidophilic Green Alga Chlamydomonas Sp.1. J. Phycol. 32, 598–601. doi:10.1111/j.0022-3646.1996.00598.x

Temizel, C., Betancourt, D.J., Tiwari, A., Zhang, M., Aktas, S.S., Quiros, F., 2016. Optimization of Enhanced Coalbed Methane Recovery With C02 Injection. Presented at the SPE Argentina Exploration and Production of Unconventional Resources Symposium, Society of Petroleum Engineers. doi:10.2118/180973-MS

Tredici, M.R., 2003. Mass Production of Microalgae: Photobioreactors, in: Richmond, A. (Ed.), Handbook of Microalgal Culture. Blackwell Publishing Ltd, pp. 178–214.

Tsai, D.D.-W., Chen, P.H., Ramaraj, R., 2017. The potential of carbon dioxide capture and sequestration with algae. Ecol. Eng. 98, 17–23. doi:10.1016/j.ecoleng.2016.10.049

Tung, H.-L., Tu, C.-C., Chang, Y.-Y., Wu, W.-T., 1998. Bubble characteristics and mass transfer in an airlift reactor with multiple net draft tubes. Bioprocess Eng. 18, 323–328. doi:10.1007/s004490050450

Ugwu, C.U., Aoyagi, H., Uchiyama, H., 2008. Photobioreactors for mass cultivation of algae. Bioresour. Technol. 99, 4021–4028. doi:10.1016/j.biortech.2007.01.046

Ullah, K., Ahmad, M., Sofia, Sharma, V.K., Lu, P., Harvey, A., Zafar, M., Sultana, S., 2015. Assessing the potential of algal biomass opportunities for bioenergy industry: A review. Fuel 143, 414–423. doi:10.1016/j.fuel.2014.10.064

U.S. Department of Energy (DOE), 2010. National algal biofuels technology roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, Washington, DC.

Vadiveloo, A., Moheimani, N.R., Cosgrove, J.J., Bahri, P.A., Parlevliet, D., 2015. Effect of different light spectra on the growth and productivity of acclimated Nannochloropsis sp. (Eustigmatophyceae). Algal Res. 8, 121–127. doi:10.1016/j.algal.2015.02.001

Van Den Hende, S., Vervaeren, H., Boon, N., 2012. Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. Biotechnol. Adv. 30, 1405–1424. doi:10.1016/j.biotechadv.2012.02.015

Vasumathi, K.K., Premalatha, M., Subramanian, P., 2012. Parameters influencing the design of photobioreactor for the growth of microalgae. Renew. Sustain. Energy Rev. 16, 5443–5450. doi:10.1016/j.rser.2012.06.013

Venkata Mohan, S., Devi, M.P., 2014. Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae. Bioresour. Technol., Special Issue: CESE 2013 & Special Issue: ICABB 2013 165, 288–294. doi:10.1016/j.biortech.2014.02.103

Vidyashankar, S., Deviprasad, K., Chauhan, V.S., Ravishankar, G.A., Sarada, R., 2013. Selection and evaluation of CO_2 tolerant indigenous microalga Scenedesmus dimorphus for unsaturated fatty acid rich lipid production under different culture conditions. Bioresour. Technol. 144, 28–37. doi:10.1016/j.biortech.2013.06.054

Walker, J., Halliday, D., Resnick, R., 2011. Halliday & Resnick Fundamentals of physics. John Wiley & Sons, Hoboken, NJ.

Wang, J., Zhou, W., Yang, H., Wang, F., Ruan, R., 2015. Trophic mode conversion and nitrogen deprivation of microalgae for high ammonium removal from synthetic wastewater. Bioresour. Technol. 196, 668–676. doi:10.1016/j.biortech.2015.08.036

Wang, X., Sun, D., Yao, T., 2016. Climate change and global cycling of persistent organic pollutants: A critical review. Sci. China Earth Sci. 59, 1899–1911. doi:10.1007/s11430-016-5073-0

Wang, Y., He, B., Sun, Z., Chen, Y.-F., 2016. Chemically enhanced lipid production from microalgae under low sub-optimal temperature. Algal Res. 16, 20–27. doi:10.1016/j.algal.2016.02.022

Wei, L., Huang, X., Huang, Z., 2015. Temperature effects on lipid properties of microalgae Tetraselmis subcordiformis and Nannochloropsis oculata as biofuel resources. Chin. J. Oceanol. Limnol. 33, 99–106. doi:10.1007/s00343-015-3346-0

Weissman, J.C., Goebel, R.P., Benemann, J.R., 1988. Photobioreactor design: Mixing, carbon utilization, and oxygen accumulation. Biotechnol. Bioeng. 31, 336–344. doi:10.1002/bit.260310409

Welter, C., Schwenk, J., Kanani, B., Van Blargan, J., Belovich, J.M., 2013. Minimal medium for optimal growth and lipid production of the microalgae *Scenedesmus dimorphus*. Environ. Prog. Sustain. Energy 32, 937–945. doi:10.1002/ep.11835

Widjaja, A., Chien, C.-C., Ju, Y.-H., 2009. Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. J. Taiwan Inst. Chem. Eng. 40, 13–20. doi:10.1016/j.jtice.2008.07.007

Wu, H., Jiang, H., Liu, C., Deng, Y., 2015. Growth, pigment composition, chlorophyll fluorescence and antioxidant defenses in the red alga Gracilaria lemaneiformis (Gracilariales, Rhodophyta) under light stress. South Afr. J. Bot. 100, 27–32. doi:10.1016/j.sajb.2015.05.017

Wu, Y.-H., Yu, Y., Hu, H.-Y., 2013. Potential biomass yield per phosphorus and lipid accumulation property of seven microalgal species. Bioresour. Technol. 130, 599–602. doi:10.1016/j.biortech.2012.12.116

Xia, J., Gong, S., Jin, X., Wan, M., Nie, Z., 2013. Effects of simulated flue gases on growth and lipid production of Chlorella sorokiniana CS-01. J. Cent. South Univ. 20, 730–736. doi:10.1007/s11771-013-1541-8

Xia, L., Rong, J., Yang, H., He, Q., Zhang, D., Hu, C., 2014. NaCl as an effective inducer for lipid accumulation in freshwater microalgae Desmodesmus abundans. Bioresour. Technol. 161, 402–409. doi:10.1016/j.biortech.2014.03.063

Xin, L., Hong-ying, H., Yu-ping, Z., 2011. Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresour. Technol. 102, 3098–3102. doi:10.1016/j.biortech.2010.10.055

Xue, S., Su, Z., Cong, W., 2011. Growth of Spirulina platensis enhanced under intermittent illumination. J. Biotechnol. 151, 271–277. doi:10.1016/j.jbiotec.2010.12.012

Yadav, G., Sen, R., 2017. Microalgal green refinery concept for biosequestration of carbon-dioxide vis-à-vis wastewater remediation and bioenergy production: Recent technological advances in climate research. J. CO_2 Util. 17, 188–206. doi:10.1016/j.jcou.2016.12.006

Yan, C., Muñoz, R., Zhu, L., Wang, Y., 2016. The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae Chlorella sp. Energy 106, 554–561. doi:10.1016/j.energy.2016.03.033

Yang, W., Zou, S., He, M., Fei, C., Luo, W., Zheng, S., Chen, B., Wang, C., 2016. Growth and lipid accumulation in three Chlorella strains from different regions in response to diurnal temperature fluctuations. Bioresour. Technol. 202, 15–24. doi:10.1016/j.biortech.2015.11.070

Yeesang, C., Cheirsilp, B., 2011. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. Bioresour. Technol. 102, 3034–3040. doi:10.1016/j.biortech.2010.10.013

Yilancioglu, K., Cokol, M., Pastirmaci, I., Erman, B., Cetiner, S., 2014. Oxidative Stress Is a Mediator for Increased Lipid Accumulation in a Newly Isolated Dunaliella salina Strain. PLoS ONE 9. doi:10.1371/journal.pone.0091957

Yoshihara, K.-I., Nagase, H., Eguchi, K., Hirata, K., Miyamoto, K., 1996. Biological elimination of nitric oxide and carbon dioxide from flue gas by marine microalga NOA-113 cultivated in a long tubular photobioreactor. J. Ferment. Bioeng. 82, 351–354. doi:10.1016/0922-338X(96)89149-5

Yoshioka, M., Yago, T., Yoshie-Stark, Y., Arakawa, H., Morinaga, T., 2012. Effect of high frequency of intermittent light on the growth and fatty acid profile of Isochrysis galbana. Aquaculture 338–341, 111–117. doi:10.1016/j.aquaculture.2012.01.005

Yusof, Y. a. M., Basari, J.M.H., Mukti, N.A., Sabuddin, R., Muda, A.R., Sulaiman, S., Makpol, S., Ngah, W.Z.W., 2011. Fatty acids composition of microalgae Chlorella vulgaris can be modulated by varying carbon dioxide concentration in outdoor culture. Afr. J. Biotechnol. 10, 13536–13542. doi:10.5897/AJB11.1602

Zeng, X., Danquah, M.K., Chen, X.D., Lu, Y., 2011. Microalgae bioengineering: From CO_2 fixation to biofuel production. Renew. Sustain. Energy Rev. 15, 3252–3260. doi:10.1016/j.rser.2011.04.014

Zhang, W., Liu, H., Sun, Y., Cakstins, J., Sun, C., Snape, C.E., 2016. Parametric study on the regeneration heat requirement of an amine-based solid adsorbent process for post-combustion carbon capture. Appl. Energy 168, 394–405. doi:10.1016/j.apenergy.2016.01.049

Zhang, X., Bai, L., Zeng, S., Gao, H., Zhang, S., Fan, M., 2017. Ionic Liquids: Advanced Solvents for CO_2 Capture, in: Budzianowski, W.M. (Ed.), Energy Efficient Solvents for CO_2 Capture by Gas-Liquid Absorption, Green Energy and Technology. Springer International Publishing, pp. 153–176. doi:10.1007/978-3-319-47262-1_7

Zhang, Y.-M., Chen, H., He, C.-L., Wang, Q., 2013. Nitrogen starvation induced oxidative stress in an oil-producing green alga Chlorella sorokiniana C3. PloS One 8, e69225. doi:10.1371/journal.pone.0069225

Zhang, Z., Huisingh, D., 2017. Carbon dioxide storage schemes: Technology, assessment and deployment. J. Clean. Prod., Special Volume on Improving natural resource management and human health to ensure sustainable societal development based upon insights gained from working within “Big Data Environments” 142, Part 2, 1055–1064. doi:10.1016/j.jclepro.2016.06.199

Zhao, B., Su, Y., 2014. Process effect of microalgal-carbon dioxide fixation and biomass production: A review. Renew. Sustain. Energy Rev. 31, 121–132. doi:10.1016/j.rser.2013.11.054

Zhou, W., Chen, P., Min, M., Ma, X., Wang, J., Griffith, R., Hussain, F., Peng, P., Xie, Q., Li, Y., Shi, J., Meng, J., Ruan, R., 2014. Environment-enhancing algal biofuel production using wastewaters. Renew. Sustain. Energy Rev. 36, 256–269. doi:10.1016/j.rser.2014.04.073

Zhou, X., Yuan, S., Chen, R., Song, B., 2014. Modelling microalgae growth in nitrogen-limited continuous culture. Energy 73, 575–580. doi:10.1016/j.energy.2014.06.058

Zimmerman, W.B., Zandi, M., Hemaka Bandulasena, H.C., Tesař, V., James Gilmour, D., Ying, K., 2011. Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae Dunaliella salina. Appl. Energy, Special Issue of Energy from algae: Current status and future trends 88, 3357–3369. doi:10.1016/j.apenergy.2011.02.013

Zwaan, B. van der, Gerlagh, R., 2016. Offshore CCS and ocean acidification: a global long-term probabilistic cost-benefit analysis of climate change mitigation. Clim. Change 137, 157–170. doi:10.1007/s10584-016-1674-5